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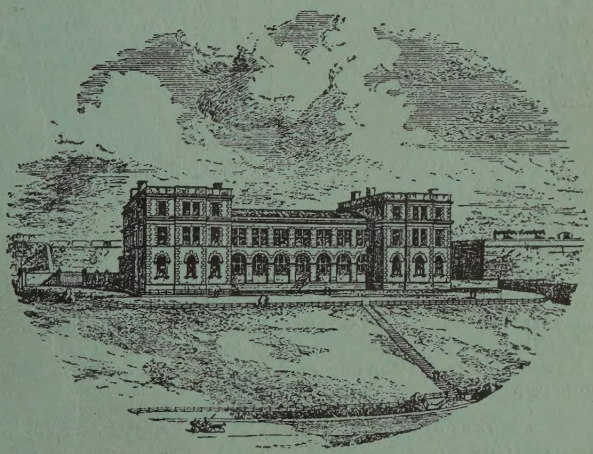

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On the Biology of *Calanus finmarchicus.* Part VI. Oxygen Consumption in Relation to Environmental Conditions.

By

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and

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With 7 Figures in the Text.

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INTRODUCTION.

VERY few observations have hitherto been made on the respiration of marine copepods and apart from the work by Pütter (1909, 1922, 1923, 1924–25) and a single observation by Ostenfeld (1913) little is known. Pütter used mixed plankton catches containing several species of copepods and other organisms in addition to phytoplankton and bacteria. By comparing the results of a number of experiments with different quantities of plankton he was able to calculate the oxygen requirements of the copepods which he divided into three groups, small, medium and large. Subsequent work, however, has shown that his results are too high.

The object of the experiments recorded in the present paper was primarily to find the food requirements of *Calanus finmarchicus.* In

addition, the influence on respiration was investigated of those conditions, which, in the sea, are subject to seasonal change. As a rule, the range of variation studied was not much beyond that which actually occurs in the sea.

Where Calanus is mentioned in this paper it should be understood that *C. finmarchicus* is referred to.

Methods.

The Calanus were taken, usually in deep water, off Garroch Head by a stramin net and the catch, suitably diluted, brought back to the laboratory in large glass jars. They were usually picked out at once, separated into males, females and Stage V and put into sea-water filtered through a coarse sintered glass filter. Males were sometimes scarce and could not always be got in sufficient numbers. No work was done on younger stages. The three classes can be distinguished by the naked eye and for the majority of the experiments they were picked out by eye examination and checked with the aid of a lens.

As a rule 120 Calanus were put in a bottle of about 170 ml. capacity and this was fitted with a two-holed rubber stopper. Through one hole a glass tube passed which projected just below the stopper; this was connected to a large reservoir of filtered sea-water, the physical or chemical condition of which was adjusted according to the experiment. The outlet tube passed through the other hole in the stopper to near the bottom of the bottle where it was covered with bolting silk to prevent the escape of the Calanus. The bottle was filled and washed through with eight to nine times its own volume of water and then samples for oxygen determination were drawn off into bottles of about 50 ml. capacity. The rubber stopper was removed and a well-ground glass stopper inserted, care being taken to exclude air bubbles. The bottle was enclosed in a dark cloth bag and submerged for a suitable time, usually four hours, in a tank kept at constant temperature by means of a thermostat. At the end of the experiment the bottle was well shaken and samples for oxygen determination withdrawn by a siphon, the inlet of which was covered with bolting silk. Oxygen determinations were made, usually in duplicate, by Winkler's method, using for the titration N/200 sodium thiosulphate. The oxygen consumed during the experiment is in all cases expressed as the amount of oxygen used by 1000 Calanus in one hour.

In a few of the earliest experiments, the method adopted was to put ten or twenty Calanus in a bottle of about 50 ml. capacity filled with sea-water, to expose this to the experimental conditions along with a control bottle without Calanus and, after a suitable period, to estimate the oxygen in these bottles. This method was discontinued because when the small bottles were used the Calanus had to be killed after each experiment.

The technique was altered to make it possible to use the same Calanus several times. In addition, by using a larger number of animals, the experimental error was reduced.

During long experiments, there were often a few Calanus lost by death or misadventure. Where possible, allowance was made for these in computing the results.

It was found that there was no measurable reduction in the oxygen content of samples of filtered sea-water at the beginning and end of an experiment so that the effect of any microplankton or bacteria passing through the filter was negligible. Moreover the duration of the experiments was too short to allow of any appreciable growth of bacteria in the bottles containing the Calanus. Thus the reduction in the oxygen content can be attributed to the Calanus alone.

A possible objection to the method used is that the Calanus remained for four hours in the same body of water which was not renewed till the end of this period. There was a progressive diminution of dissolved oxygen and an accumulation of carbon dioxide and waste products in the course of each experiment. Except, however, in those cases dealing specifically with low oxygen content (page 10) and a few other cases mentioned in the text, the oxygen used was on an average not more than a fifth of that present at the beginning. Owing to the difficulty of making sufficiently accurate analyses of the carbon dioxide content of sea-water no estimations of the respiratory quotient were made.

The Calanus were not anæsthetised and the Stage V Calanus usually swam about actively ; males and females were more sluggish and remained on or near the bottom of the bottle. It was thought, however, that it would be desirable to obtain a value for their respiration near the normal value in the sea rather than the basal oxygen consumption obtainable by anæsthetising the animals. Since good duplicates could be obtained it was felt that this was justified.

Oxygen Consumption under Standard Conditions.

In measurements of the oxygen consumption time must be taken into account. It is frequently found that during the first few hours after capture the oxygen consumption is considerably above the values obtained subsequently. This is shown in the experiments in Figure 1 and Table I. In the March experiment (Figure 1, B) male and female Calanus were picked out as soon as possible after capture and subjected to consecutive four-hourly periods of experimental conditions at constant temperature for thirty-six hours. This was followed by later estimations at 48 and 72 hours after capture. The females showed a sharp fall from the first to the second four-hourly period, followed by a slow irregular fall up to thirty-six hours after capture. The values remained approximately

the same at 48 and 72 hours, and duplicate bottles of female Calanus, not used before, gave at 30 and 73 hours after capture values not far removed from those given by the experimental Calanus. The parallel experiment on males was not begun early enough to show the rapid initial fall and subsequent results were irregular, showing after 48 hours a rise to a value higher than the original value found. A similar fall is shown in an earlier experiment in February (Figure 1, A, and Table I) in both males and females, but on this occasion there was a longer time between capture and the beginning of the experiment and the initial rapid fall is

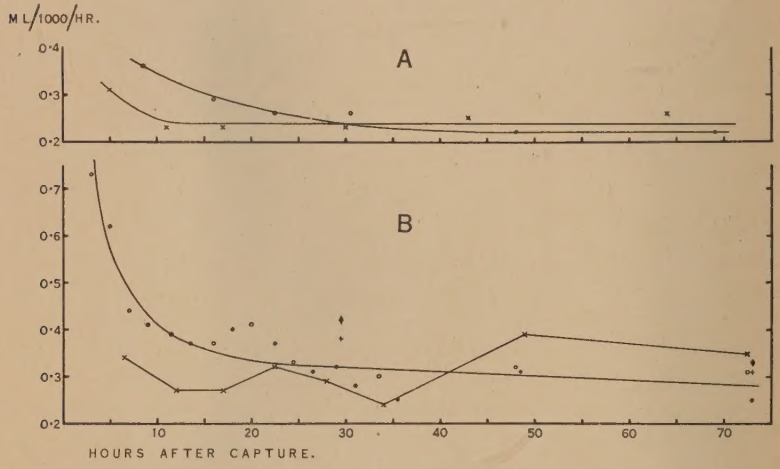

FIG. 1.—The fall in oxygen consumption with time.
A. 25–28.2.31 B. 1–4.3.32
× Males. ° Females. ⧫ + Control Females.

not shown. This rapid fall in respiration is most marked in females and less marked in males while Stage V, which were used in only a few experiments of this type, showed no fall. The fall is apparently more definite in winter than in summer.

Two explanations for this rapid initial fall have been put forward in similar work on respiration. In the first place the animals may have been so much disturbed by the processes of capture and picking out that their metabolism was greatly increased and only gradually fell again to normal values. It is known from work on fish (Keys, 1930) that values for their respiration are above normal for some time after they have been put under experimental conditions. In the second place, the keeping of Calanus in the laboratory and their exposure to varying conditions in small bottles is injurious to them. Usually in the course of a prolonged experiment several died, and it seems probable that the metabolism of the whole

number, and therefore their respiration, decreases gradually. Kreps (1929) working on the respiration of *Balanus crenatus* found a gradual slight fall in the respiration from day to day and attributed this to the unfavourable conditions of a laboratory. Calanus kept in the usual filtered water showed no difference in respiration from others given a

TABLE I.

Fall in Respiration with Time.

120 Calanus in each bottle. Exposed 4 hours at 10° C.

25–28.2.31

Males.		Females.		
Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.	Mean.
5·0	0·31	8·5	0·34	0·36
			0·38	
11·0	0·23	16·0	0·28	0·29
			0·29	
17·0	0·23	22·5	0·26	0·26
			0·25	
30·0	0·23	30·5	0·24	0·26
			0·27	
43·0	0·25	48·0	0·21	0·22
			0·23	
64·0	0·26	69·0	0·23	0·22
			0·21	

1–4.3.32

Males.		Females. A.		Females. B.	
Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.
6·5	0·34	3·0	0·73	5·0	0·62
12·0	0·27	7·0	0·44	9·0	0·41
17·0	0·27	11·5	0·39	13·5	0·37
22·5	0·32	16·0	0·37	18·0	0·40
28·0	0·29	20·0	0·41	22·5	0·37
34·0	0·24	24·5	0·33	26·5	0·31
49·0	0·39	29·0	0·32	31·0	0·28
72·5	0·35	33·5	0·30	35·5	0·25
		48·0	0·32	48·5	0·31
		72·5	0·31	73·0	0·25
		*29·5	0·38	*29·5	0·42
		*73·0	0·31	*73·0	0·33

supply of phytoplankton. This indicates that the Calanus, under the conditions of these experiments, were not suffering from a lack of food.

A possible explanation of the fall in respiration in Calanus is that shortly after capture they were exposed to daylight for one or more hours. As is shown on page 14 this has the effect of raising their metabolism considerably and it might be that the observed fall is a result of initial abnormally high consumption caused by exposure to light after capture.

* Females not used before (see text).

There are, however, several facts which contradict this explanation. In the first place Stage V Calanus, although as sensitive to light as adults, show no definite fall. Secondly, Calanus whose respiration has been increased by exposure to light show a rapid fall to normal or sub-normal values in the dark (see page 17). Finally, in an experiment designed to test this point, in which the fall in respiration of male and female Calanus caught in the light and in the dark was compared, there was no significant difference.

The Effect of Different Environmental Conditions.

Temperature.

That Calanus can survive over a considerable range of temperature was shown by experiments in which they were subjected to gradually rising temperatures until the lethal point was reached. The first of these experiments was done in July, 1930, and 100 each of females and Stage V were used, distributed in a number of bottles of suitable size. The bottles were immersed in the experimental tank at 17° C. in the usual way and every hour the Calanus were examined and the temperature raised by 1° C.

All the Calanus remained quite healthy up to 22° C. when the females began to get sluggish and did not swim even if they were shaken up. At 24° C. two of the females had folded antennæ, usually a sign of distress, and the Stage V had become inactive. At 26° C. most of the females were apparently dead; half of all the Calanus were taken out and allowed to cool. At 27° C. the Stage V were apparently dead and the experiment was stopped. By the next morning, of those taken out at 26° C., no females and 7 Stage V had recovered and of those taken out at 27° C., 1 female and 3 Stage V had recovered. Calanus raised suddenly to temperatures of 21° C. and 25° C. behaved similarly to those raised gradually. The experiment was repeated in April, 1932, with the same general results except that the lethal temperature was about 2° C. lower. Of 50 taken out at 24° C. and allowed to cool, 3 females and 5 Stage V recovered and of 50 taken out at 25° C. no females and 1 Stage V recovered. Males were also used on this occasion but they looked unhealthy from the beginning of the experiment, and all but one were dead at 24° C.

The experiments show that male and female Calanus are less resistant to high temperatures than Stage V, and that the lethal temperature is higher in summer than in winter. This difference is probably to be ascribed to the difference in the temperature conditions under which the Calanus used in the two experiments developed. Those of the April experiment were Calanus of the first brood and developed when the sea was at its coldest while those of the July experiment, Calanus of a late summer brood, had much warmer conditions throughout their lives.

The lethal temperature for Calanus has also been measured by Huntsman and Sparks (1925). They found that Calanus taken from the sea at temperatures of 6–10° C. died between 26·5° C. and 29·5° C. This is a limit apparently higher than ours, but the temperature was raised rapidly (about 1° C. in 5 minutes) and the animals may have been able to survive such temperatures for a short time. Huntsman and Sparks do not record

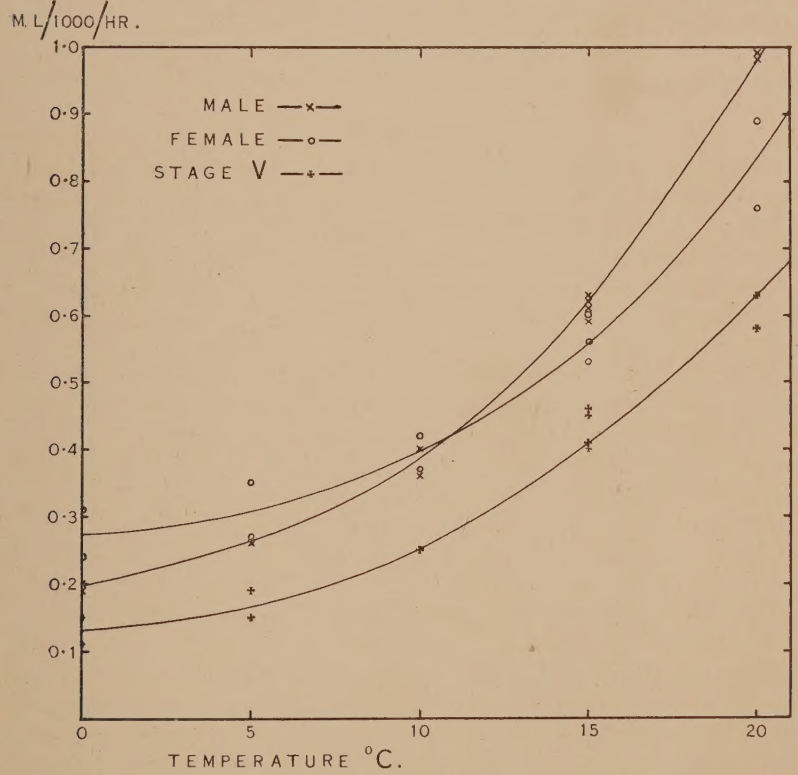

Fig. 2.—The effect of temperature on oxygen consumption. 13–14.8.31.

any difference between summer and winter Calanus. Brown (1929), however, studying Cladocera, has found that northern species and those with spring and autumn maxima have a lower lethal temperature than southern species and those with summer maxima.

The effect of temperature on oxygen utilisation by Calanus was measured at intervals of 5° C. from 0° C. to 20° C. The respiration was first measured at 10° C. or 15° C. (according to the time of year), and this was followed by successive experiments from 0° C. to 20° C., the initial

temperature being repeated to find if respiration had reached a steady value. The results of a typical experiment (in August, 1931) are shown in Figure 2 and Table II. The Calanus were allowed to stand for over 18 hours before the beginning of the experiment and the initial fall was thus avoided. The oxygen consumption at 0° C. is only about half that at 10° C. but above this the curves rise more rapidly and the oxygen consumption at 10° C. is less than half that at 20° C. Oxygen utilisation is higher in adults than in Stage V. In this and in one other experiment the values for males lay on a steeper curve, beginning lower and ending higher than those for females, but in most

TABLE II.

Effect of Temperature on Respiration. 13–14.8.31.

About 120 Calanus in each bottle, exposed for 4 hrs. Two bottles used for each stage.

	Males.		Females.		Stage V.	
T. in °C.	O_2 used in ml./1000/hr.	Mean.	O_2 used in ml./1000/hr.	Mean.	O_2 used in ml./1000/hr.	Mean.
15	0·61	0·62	0·60	0·58	0·41	0·41
	0·63		0·56		0·40	
0	0·20	0·20	0·31	0·28	0·15	0·13
	0·19		0·24		0·11	
5	0·26	0·26	0·35	0·31	0·19	0·17
	0·26		0·27		0·15	
10	0·36	0·38	0·42	0·40	0·25	0·25
	0·40		0·37		0·25	
15	0·59	0·61	0·60	0·57	0·45	0·46
	0·62		0·53		0·46	
20	0·98	0·99	0·89	0·83	0·58	0·61
	0·99		0·76		0·63	

experiments there was very little difference between them. Above 20° C. the Calanus were close to their lethal temperature and in one experiment where the respiration was measured at 25° C. there was no further increase in respiration and the Calanus were all moribund at the end of 4 hours. Indications that even at 20° C. there may be a harmful effect was shown only in one experiment, in March. At these high temperatures the oxygen consumed amounted in some cases to about a third of that initially present. The oxygen content of the bottle, however, was never so low as to affect the respiration of the Calanus.

Similar experiments were done at different times of the year and the sizes of the Calanus measured. The value for the oxygen consumption at 10° C. may vary by as much as 0·2 ml. in different experiments. The differences show no relation to size except that Stage V Calanus, which are always smaller than adults, have always a lower oxygen consumption.

It is interesting that in the early part of the year, Stage V have about the same weight as adults and in summer are considerably heavier (Orr, 1934 ; Marshall, Nicholls and Orr, 1934). In spite of this, and the fact that they are always more active, their oxygen consumption remains consistently lower. Moreover there is no consistent difference between the consumption in summer and winter for Stage V Calanus, although they are much heavier in summer than in winter.

As has been found by other workers (Ege and Krogh, 1915–16 ; Bělehrádek, 1930), the increase in oxygen consumption with temperature does not follow van't Hoff's law.

Hydrogen-ion concentration.

It is well known that changes in hydrogen-ion concentration are in many cases of importance to animal life. The changes in sea-water are small compared with those in fresh water, but it has been shown (Powers, 1930) that even the changes in the sea may be of importance. To find if these changes had any effect, Calanus were put in sea-water the pH value of which ranged from 6·7 to 8·5. The pH value was lowered by bubbling carbon dioxide through the sea-water and raised by the addition of dilute sodium hydroxide. After two days' exposure to pH 6·7 the Calanus were apparently unharmed and in no experiment was there evidence of any injurious effect at high values. Similarly it was found that the effect of changes in hydrogen-ion concentration from pH 7·3 to pH 8·5 had little

TABLE III.

Effect of pH on Respiration. 30.4.32.

About 100 female Calanus in each of the six bottles, exposed for 4 hrs. at 12° C.

pH.	O_2 used in ml./1000/hr.	Mean.
8·08	0·39	0·38
	0·37	
8·47	0·39	0·39
	0·39	
7·40	0·35	0·36
	0·37	

or no effect on the respiration of male, female or Stage V Calanus. In several cases there was a small but continuous fall from the beginning to the end of a series of experiments. When, however, separate lots of Calanus (females) were used for measurement of the respiration (Table III) at normal, low and high pH values, this fall was not shown, so that it may safely be concluded that the effect of pH change on the respiration of Calanus is negligible within the limits studied.

Oxygen content.

Variations in the oxygen content of the sea may at times be quite large. While raised oxygen content is not likely to harm Calanus, a reduction below a critical value will be lethal. This was demonstrated by an experiment in August, 1931. At this time of the year the normal oxygen content is about 6 ml. per litre. The oxygen content was reduced by passing through the sea-water hydrogen washed successively with alkaline lead acetate, silver nitrate and distilled water to remove any traces of injurious gases. That the washing was successful was shown by re-oxygenating the water by agitation with air and leaving a number of Calanus in it for 48 hours during which time they were not harmed. An experiment was then done in which 100 each of male, female and Stage V Calanus were used at 5° C. and 15° C. and the oxygen content lowered by stages to about 3·3, 2·5, 1·4 and (for Stage V) 0·7 ml. per litre. The Calanus were left at each concentration for two hours and examined every hour.

Males at 15° C. Six were dead after two hours at 3·3 ml. per litre; after an hour at 2·5 ml. per litre the rest were apparently normal, but after two hours they were definitely sluggish and 20 more were dead. When the oxygen was reduced to 1·4 ml. per litre, all died within an hour.

Females at 15° C. After two hours, even at 3·3 ml. per litre, they were sluggish but survived one hour at 2·5 ml. per litre. After two hours at this oxygen content nearly half were dead and only 2 survived after one hour at 1·4 ml. per litre. These did not survive the second hour.

Stage V at 15° C. These were definitely more resistant to low oxygen tensions. They were sluggish after two hours at 2·5 ml. per litre; most were moribund after two hours at 1·3 ml. per litre and all were dead after one hour at 0·7 ml. per litre.

At 5° C. the resistance to lowered oxygen content was increased.

Males at 5° C. Five were dead after two hours at 3·3 ml. per litre; 4 more were dead and the remainder were sluggish after two hours at 2·4 ml. per litre; only one survived two hours at 1·4 ml. per litre, the remainder having died within an hour.

Females at 5° C. These became moribund at 1·4 ml. per litre but a few were alive after two hours at this low value.

Stage V at 5° C. These were sluggish throughout but the mortality was low until the oxygen was reduced to 0·7 ml. per litre when almost all were dead within one hour.

A low oxygen concentration affects the respiration of Calanus directly as is shown in Figure 3 and Table IV. This experiment was done, using the earlier method (see page 2), on females and Stage V at 15° C. and 5° C. With females, when the oxygen concentration fell below about 3 ml. per litre, the consumption also fell even though at the end of four hours they

had not used up more than about 20% of the oxygen initially present. At the end of this experiment over 90% of the Calanus were still fairly active. At a lowered concentration (2·2 ml. per litre) the fall in consumption was still more marked but towards the end of the experiment the Calanus were dying rapidly. The final oxygen content in this experiment was 1·8 ml. per litre. The Calanus were killed off completely at an oxygen concentration of about 1 ml. per litre. The Stage V Calanus were more resistant and their respiration was little affected even at 2·2 ml. per litre ; at 1 ml. per litre, however, the majority were dead at the end of four hours.

When the experiment was repeated at 5° C. (Table IV) the resistance of

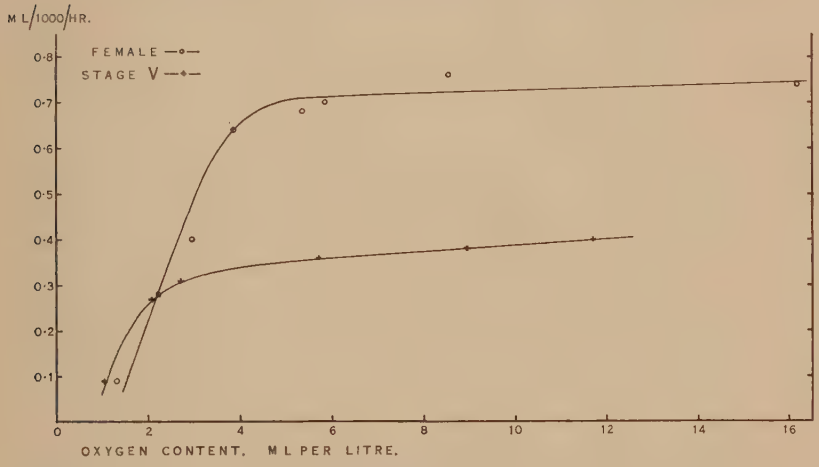

Fig. 3.—The effect of oxygen content on the oxygen consumption of female and Stage V Calanus, at 15° C.

both females and Stage V was greater ; a larger number survived at low oxygen concentrations and the drop in consumption was not so marked.

Raising the oxygen content even as high as 19 ml. per litre had no effect on the respiration. The absence of any injurious gases in the commercial oxygen (washed with distilled water) used to raise the oxygen content was demonstrated by estimating the respiration of Calanus in a sample of water of which the oxygen content had first been raised to 18 ml. per litre and then reduced to normal by agitation with air. The oxygen consumption was the same as in normal sea-water.

It is apparent from these experiments that the respiration of Calanus is unaffected by changes in dissolved oxygen concentration except at and below about 3 ml. per litre.

Similar conclusions were reached by Henze (1910) who states that in cold-blooded animals with good circulation and branchial respiration the oxygen consumption is, within wide limits, independent of the oxygen

TABLE IV.

Effect of Oxygen Content on Respiration. 31.7.30–7.8.30.

Oxygen used, in ml. per 1000 per hour based on triplicate samples of about 20 Calanus.

15° C.				5° C.		
Females		Stage V				
O_2 content in ml./litre.	O_2 used in ml./1000/hr.	O_2 content in ml./litre.	O_2 used in ml./1000/hr.	O_2 content in ml./litre.	O_2 used in ml./1000/hr. Females.	Stage V.
16·17	0·74	11·69	0·40	2·04	0·28	0·16
8·54	0·76	8·94	0·38	1·21	0·09	0·09
5·85	0·70	5·71	0·36			
5·36	0·68	2·72	0·31			
3·84	0·64	2·06	0·27			
2·94	0·40	1·03	0·09			
2·21	0·28					
1·31	0·09					

tension. Hyman (1930) found the same to hold in planarians and the oxygen tension below which respiratory activity decreased (3 ml. per litre) was much the same as that found in Calanus.

Salinity.

Fluctuations in salinity in the sea are not very great except in coastal waters but this is a factor of the environment which may sometimes affect Calanus. If the salinity of the water in which Calanus are living is

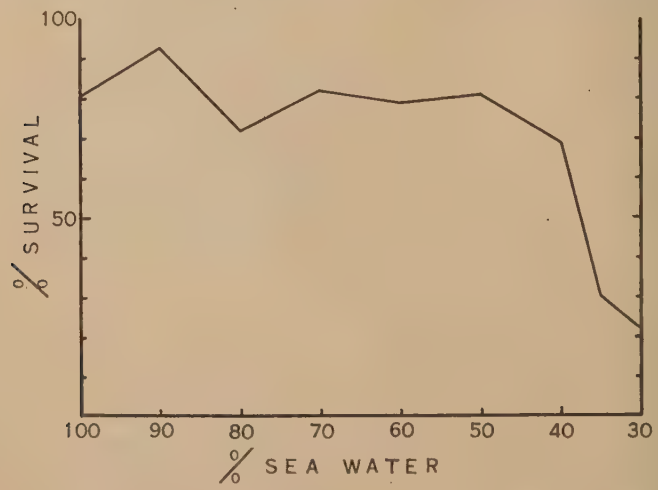

Fig. 4.—The effect of reduced salinity on the survival of Stage V Calanus.

changed in the course of two or three hours from normal (about 34°/$_{oo}$ for this area) to about 66% sea-water, they die within a short time. If, however, the change is accomplished more gradually, they can become acclimatised. This is shown by an experiment carried out from May 24th to June 9th, 1932. A thousand Stage V Calanus were picked out and put by fifties into beakers. Two hundred of these Calanus were kept in normal sea-water, the rest were then transferred at once to 95% sea-water, that

TABLE V.

EFFECT OF LOWERED SALINITY ON THE SURVIVAL OF FEMALE CALANUS.

	100%	90%	80%	70%	60%	50%	40%	35%	30%
Salinity.	S.= 33·91°/$_{oo}$		S.= 27·18°/$_{oo}$			S.= 16·93°/$_{oo}$		S.= 11·98°/$_{oo}$	S.= 10·22°/$_{oo}$
% survival	81	93	72	82	79	81	69	30	22

is, to freshly drawn sea-water diluted in the correct proportions with glass distilled water. The water was changed daily and at the same time the salinity was reduced by 5%. The Calanus in the first four beakers remained in normal sea-water (about 33·9°/$_{oo}$) throughout; the Calanus in the next pair of beakers were brought down to 90% sea-water (in two steps) and remained at 90% for the rest of the experiment; the Calanus in the next pair were reduced to 80% (in four steps) where they remained,

TABLE VI.

EFFECT OF LOWERED SALINITY ON RESPIRATION. 6.6.32.

About 80 female Calanus in each bottle exposed for 4 hrs. at 15° C.

Salinity.	100% S.=33·91°/$_{oo}$	80% S.=27·16°/$_{oo}$	50% S.=16·97°/$_{oo}$
O_2 used in ml./1000/hr.	0·37 0·37	0·38	0·25

and so on. By the twelfth day the Calanus in the last four beakers were in 35% sea-water (S=12·0°/$_{oo}$) and the mortality was high. It was noticed that as the salinity was reduced the Calanus became less active. The Calanus in two of the last four beakers were kept in 35% sea-water and those in the other two reduced to 30% at which the majority of those remaining died. On June 6th some of the Calanus were used for a respiration experiment and on June 9th the experiment was stopped and the survivors counted. The results are shown in Figure 4 and Table V. There was no definite injury and the death rate was irregular and not very high until 40% sea-water was reached. Below this the animals died off

rapidly. The mortality at the high salinities is to be accounted for by the long duration of the experiment.

The respiration values (Table VI) show that, whereas the Calanus in 80% sea-water (S=27·2‰) had an oxygen consumption as high as the Calanus in normal sea-water, those in 50% (S=17·0‰) had one which was definitely lower. Thus Calanus can become acclimatised to salinities as low as 12–17‰ although respiration is reduced before these values are reached.

In several marine invertebrates (*Nereis* sp., *Procerodes* (*Gunda*) *ulvæ*) a decrease in salinity causes at least an initial increase in respiratory activity (Beadle, 1931), which is contrary to the effect found for Calanus.

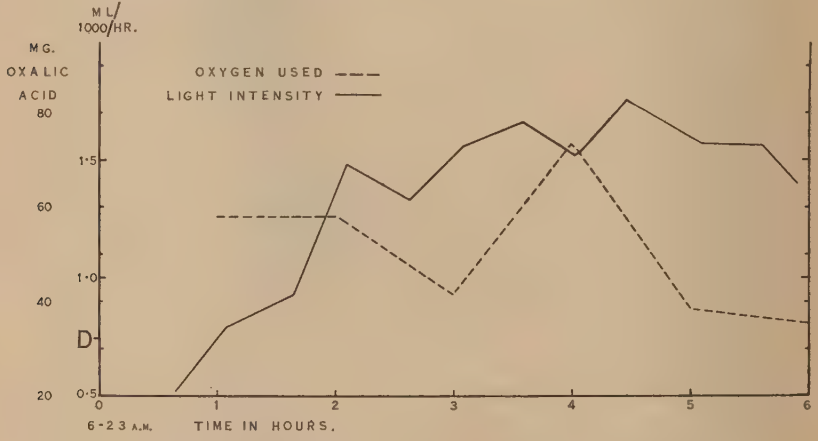

Fig. 5.—The oxygen consumption of female Calanus in bright sunshine compared with the light intensity over the same period. 10.5.32. D= Value for respiration in the dark.

Kreps (1929), however, working on Balanus found that although at salinities from 12‰ to 35‰ respiration was almost independent of salinity, below 12‰ it decreased.

Light.

When exposed outside in ordinary clear glass bottles, there is no apparent increase in the activity of Calanus but there is a very considerable increase in oxygen uptake. In bright diffuse light or in sunshine, the respiration may be even double what it is in the dark (Table IX). The increase is not due to a rise in temperature for care was taken to keep all the experimental bottles at a constant temperature. The light intensity at which this increase in respiration was obtained is comparatively low. Calanus which were exposed out of doors shaded from direct sunlight

gave values as high as those which were fully exposed during the same time. Similar high values were obtained also on a cloudy day. Exposure to artificial light* or to the diffuse light indoors in front of a north window had no appreciable effect.

To find the effect of continued exposure to sunlight, 12 bottles containing female Calanus were exposed for periods from one up to six hours on May 10th, 1932, two bottles being removed each hour (Figure 5 and

TABLE VII.

The Effect of Sunlight on the Respiration of Female Calanus at about 12° C.

Experiment began 6.23 a.m. G.M.T. 10.5.32.

Time exposed in hours.	Actual consumption of O_2 in ml./100 Calanus.	O_2 used in ml./1000/hr.	Mean.	†Calculated consumption in successive hours.
1	0·114	1·14	1·26	1·26
	0·137	1·37		
2	0·254	1·27	1·26	1·26
	0·247	1·24		
3	0·343	1·14	1·15	0·93
	0·348	1·16		
4	0·543	1·36	1·31	1·57
	0·501	1·25		
5	0·573	1·15	1·17	0·87
	0·594	1·19		
6	0·667	1·11	1·16	0·81
	0·723	1·21		
3 (dark)	0·220	0·73		
3 ,,	0·218	0·73	0·74	
3 ,,	0·228	0·76		
3 ,,	0·216	0·72		

Tables VII and VIII). Only 50 Calanus were put in the bottles which were to be exposed for four hours or more so that they should not suffer from lack of oxygen. The remainder contained 100. There were, besides, four controls in the dark. The temperature during this experiment was 12° C. The light intensity (measured by Anderson and Robinson's method (1925)) rose during the first two hours and remained more or less constant during the rest of the experiment. The oxygen consumption although it fluctuated considerably showed no relation to the variations in

* The source of artificial light was a 110 V. 60 W. Phillips " Argenta " gas-filled lamp with a parabolic reflector, at a distance of about 25 cm.

† Obtained by subtracting the sum of the means for the preceding hours from the actual consumption at any given hour.

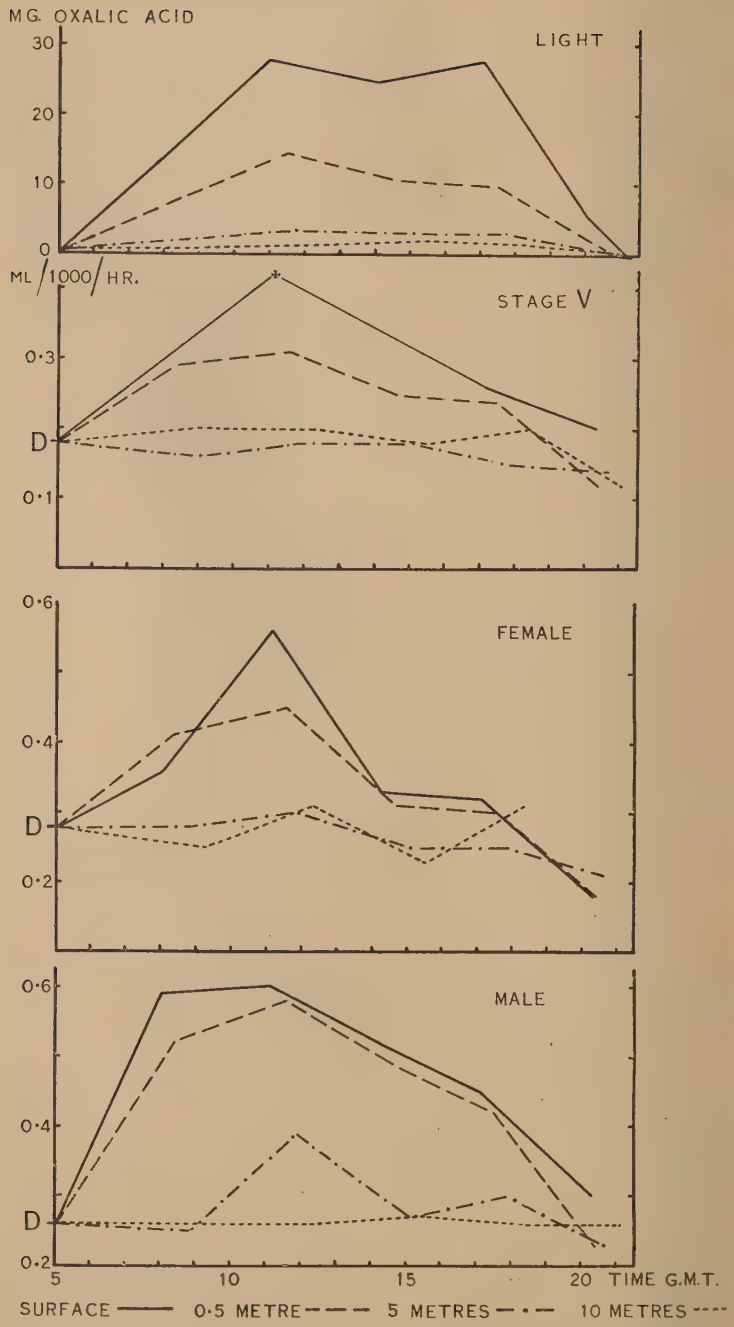

FIG. 6.—The oxygen consumption of Calanus at different depths in the sea and its relation to light intensity. 13.4.32. D=Value for respiration in the dark.

light intensity, even at the beginning. This indicated that, beyond a certain point, light intensity has no further effect on respiration. After the experiment all the bottles which had been exposed to the light were kept in the dark for three hours or (with bottles containing only 50 Calanus) six hours, and the respiration measured. In all cases but one, the value was much below that of the controls in the dark, suggesting that the exposed Calanus had been injured by light.

It will be noticed that the value for respiration in the dark was in this experiment unusually high (0·74 ml. at 12° C.). The Calanus were taken in a surface tow-netting during the day and the metabolism of those living at the surface is probably higher than that of those living in deep water.

TABLE VIII.

Light Measurements on 10.5.32.

Time in hours.	Mg. oxalic acid used per hour.
0·65	20·8
1·08	34·6
1·65	41·5
2·10	68·8
2·63	61·5
3·08	73·0
3·58	78·1
4·03	70·9
4·57	81·4
5·08	73·5
5·60	73·2
5·90	64·9
6·48	76·7

Since light must therefore have an effect under natural conditions, Calanus were exposed at different depths in the sea to find out to what depth its influence could be detected. The bottles were put in small wire cages and these were attached to a buoyed and anchored rope. The surface bottles were on a small float attached to the buoy.

In a preliminary experiment on males on April 5th, 1932, the dark value was 0·28 ml., that at the surface was 0·64 ml. and that at 5 metres was 0·36 ml., showing that a slight effect may be noticed even at 5 metres. A long experiment was then carried out to measure the variations in the respiration of male, female and Stage V Calanus at different depths throughout a day. Bottles were exposed at the surface, 0·5, 5 and 10 metres for five periods of about three hours from 5 a.m. onwards. Since washing through the bottles with fresh sea-water at the beginning of each period took a considerable time, two sets of bottles had to be used for each depth. Thus one set was exposed from 5 a.m. to 8 a.m., 11 a.m. to 2 p.m. and 5 p.m. to 8 p.m. while the second set was used from 8 a.m.

to 11 a.m., and from 2 p.m. to 5 p.m. The different sets of bottles are indicated in Table IX by letters. The results are shown in Figure 6 and Tables IX and X. Although the Calanus were caught on April 12th and were left overnight after picking out, it will be seen that there is a fall in respiration in most bottles from one period of exposure to the next. This may be caused in part by the fall with time and in part by

TABLE IX.

The Respiration of Calanus at Different Depths in the Sea During the Day. 13.4.32.

About 100 Calanus in each bottle at about 7·5° C. Secchi disc reading 5·5 m.
O_2 used in ml./1000/hr.

Time of exposure.	Males.	Females.	Stage V.
	Surface.		
5·05— 8·05	A 0·59	A 0·36	Lost
7·50—11·10	E 0·60	E 0·56	E 0·42
10·55—14·15	A 0·52	A 0·33	Lost
14·05—17·10	E 0·45	E 0·32	E 0·26
17·00—20·20	A 0·30	A 0·18	D 0·20
	½ metre.		
5·25— 8·25	B 0·52	B 0·41	B 0·29
8·15—11·35	F 0·58	F 0·45	F 0·31
11·20—14·40	B 0·49	B 0·31	B 0·25
14·30—17·30	F 0·42	F 0·30	F 0·24
7·20—20·25	B 0·23	B 0·18	B 0·12
	5 metres.		
5·35— 8·50	C 0·25	C 0·28	C 0·16
8·35—11·55	G 0·39	G 0·30	G 0·18
11·45—15·05	C 0·27	C 0·25	C 0·18
14·50—17·55	G 0·30	G 0·25	G 0·15
17·40—20·40	C 0·23	C 0·21	C 0·14
	10 metres.		
5·55— 9·15	D 0·26	D 0·25	D 0·20
9·00—12·20	H 0·26	H 0·31	H 0·20
12·00—15·30	D 0·27	D 0·23	D 0·18
15·05—18·20	H 0·26	H 0·31	H 0·20
18·05—21·05	D 0·26	Lost	E 0·12

the injurious effect of exposure to light. Both these factors complicate the results. The light intensity, measured over the same time, showed that there was a rapid increase up to a maximum at 11 a.m., a slight decrease at 2 p.m. and a rise to a maximum value again at 5 p.m. after which it fell off rapidly. It will be seen that the respiration values from 5 p.m. to 8 p.m. in all cases, and those at 5 and 10 metres throughout the day, with one exception, can be taken as equivalent to dark values. The exception is in males at 5 metres from 8–11 a.m. when there was a slight increase. In all cases the maximum values at the surface and 0·5 metre were from 5 to 8 a.m. and from 8 to 11 a.m. and the two were sometimes much the same in spite of the difference between the light

intensities at these depths. Figure 6 shows that the light was equally bright from 11 a.m. to 5 p.m. but in spite of that the respiration fell off. It must be remembered, however, that all the Calanus were then being used for the second time. The males showed the effect of light most clearly and the females and Stage V showed it to a lesser degree. The light during the brightest hours was therefore sufficient to affect Calanus at the surface

TABLE X.

LIGHT MEASUREMENTS ON 13.4.32.

Time of exposure.	Mg. oxalic acid used.	Mg. per 3 hours.
	Surface.	
5·45— 7·56	9·7	13·4
8·00—11·00	27·7	27·7
11·00—14·05	25·2	24·6
14·07—17·03	27·0	27·6
17·05—20·05	5·2	5·2
	½ metre.	
6·10— 8·27	5·9	7·8
9·25—11·30	10·0	14·4
12·30—14·40	7·7	10·6
15·40—17·30	5·9	9·7
18·30—20·30	0·7	1·0
	5 metres.	
6·10— 8·45	1·7	2·0
8·45—11·45	3·3	3·3
11·45—15·05	3·3	3·0
15·05—17·45	2·7	3·0
17·45—20·45	0·2	0·2
	10 metres.	
5·54— 9·00	0·9	0·9
9·00—12·15	1·4	1·3
12·15—15·25	2·1	2·0
15·25—18·15	1·4	1·5
18·15—21·06	0·2	0·2

and at 0·5 metre, but at 5 metres and 10 metres there was little or no change.

Light may have an effect greater than that indicated in the above experiments since the glass of the bottles absorbs a certain amount, particularly the ultra-violet which according to Klugh (1929) is lethal to Calanus (see p. 21).

All the foregoing light experiments, with the possible exception of that on May 10th, were done on the first brood which is occasionally found near the surface (Marshall, Nicholls and Orr, 1934). Stage V Calanus from a summer brood were used for a similar respiration experiment in the sea in August at the surface, 0·5, 2·3, 5 and 10 metres (Figure 7 and Table XI). There was a marked increase in respiration at the surface and one slightly

smaller at 0·5 and 2·3 metres while at 5 metres there was an increase but so small that it was not much beyond experimental error.

It may thus be stated that light has no effect on respiration, or at least none that can be measured, below 5 metres.

One might expect the influence of light on the behaviour of Calanus in the sea to be reflected in the response of Calanus to light under experimental conditions, but it is difficult to find any quantitative relation

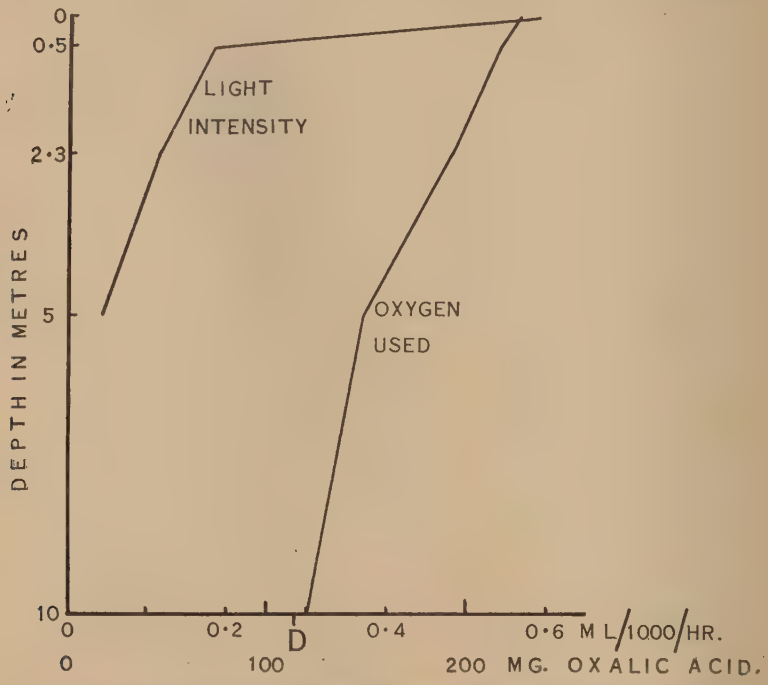

FIG. 7.—The oxygen consumption of Stage V Calanus at different depths in the sea from 10·40—14·40 G.M.T., in relation to light intensity. 22.8.32. D=Value for respiration in the dark.

between them. Huntsman (1925) has shown that light is injurious by keeping numbers of Calanus on the laboratory roof in unshaded, shaded and completely darkened jars. Those in full light all died within two or three days and only those in the dark survived for any length of time. He found that light had an equally injurious effect on many other marine animals. Our experiments on the effect of light on the respiration of Calanus lasted at most eight hours and although they were not apparently injured yet the low value of their subsequent respiration in the dark indicates that they were not unharmed. In October, in the course of

another experiment, a hundred Calanus were exposed to diffuse daylight out of doors for the greater part of the day, but after four days they all died. Even when they were given only two hours exposure daily, the death rate was much higher than when kept indoors. Klugh (1929, 1930) has shown that the lethal effect is due chiefly to the ultra-violet component of sunlight and that shallow water organisms are resistant to this. Harvey's work (1930), besides confirming Huntsman's results, showed that the rate of heart beat was decreased on exposure to sunlight and that blue light was most injurious. He dealt only with the visible rays. Klugh's results were confirmed by an experiment in which the respiration of female Calanus in plain glass bottles was compared with the respiration in bottles of Uviol glass (transparent to light of short wave length).

TABLE XI.

THE RESPIRATION OF STAGE V CALANUS AT DIFFERENT DEPTHS IN THE SEA FROM 10.40–14.40 G.M.T. IN BRIGHT SUNSHINE. 22.8.32.

Secchi disc reading >10 m.

Depth in metres.	O_2 used in ml./1000/hr. during exposure.	Light. Mg. oxalic acid used during exposure.
0	0·56	234·6
	0·57	
0·5	0·54	72·5
2·3	0·48	46·0
5·0	0·37	17·2
10·0	0·30	
Dark	0·30	
,,	0·26	

Although there was no significant difference in the figures for respiration, the mortality was much higher in the Uviol glass bottles.

Calanus does not normally live near the surface during daylight, but an interesting point is raised by their presence at certain times of the year close to the surface or actually in the surface film. When exposed thus to a high light intensity, their oxygen consumption must be greatly increased. Such a distribution would not be expected from Huntsman's and Klugh's results. Lepeschkin (1931) states that while excess of ultra-violet light may be harmful, small quantities protect copepods (*Paracalanus parvus*, *Oncœa venusta* and *Euterpe acutifrons*) from the lethal effect of the visible rays.

CALANUS IN RELATION TO ITS ENVIRONMENT.

Planktonic organisms as a rule have a wide range of distribution and *Calanus finmarchicus* is no exception. Its centre of greatest abundance

is in the North Atlantic, north of 55° N. (Farran, 1911) although it has been recorded from the Azores. It extends eastwards into the Norwegian and North Seas and it is found in the western part of the Baltic but it is less abundant in the southern North Sea and English Channel. In the western North Atlantic it is common as far south as the Gulf of Maine. It is also fairly common in the coastal waters off the west coast of North America as far south as California. Records are lacking for the Pacific as a whole but it has been found to the south of New Zealand and in the Great Australian Bight. It is recorded from the waters of South Africa, the Red Sea, the Mediterranean and the Black Sea.

Calanus normally inhabits water below 15° C. and we have seen from the experimental results that it is killed at temperatures from 25° C. to 30° C. That it can, however, become acclimatised is indicated by the records of its occurrence in the Red Sea although it is apparently not abundant there. The specimens found in warm seas are smaller than those from the North Atlantic.

The changes in pH value in the sea are relatively small and, as has been mentioned, are not likely to affect Calanus. In the Baltic where a large range in pH value is found, Calanus is not present but this is perhaps because of the low salinity.

Calanus in the laboratory is able to tolerate only a moderately low oxygen content but the lower limit is dependent on temperature. The fluctuations in oxygen content in the open sea are small, but in certain areas very low values are met with in deep water. Loch Striven and the Black Sea are examples of such areas. In late summer when Calanus is abundant in the deep water of Loch Striven the oxygen content at the bottom occasionally falls to 2 ml. per litre (Marshall and Orr, 1927). This is near the lethal limit for Calanus as found experimentally, but probably such low values do not persist for long. It is more surprising to find Calanus recorded in the Black Sea at the limiting depth for plankton in February where the oxygen content is below 1 ml. per litre, and the temperature is about 7° C. (Nikitin, 1931).

According to Farran (1911) Calanus is abundant only in water where the salinity of the sea is 35·3‰ or less. Very low salinities are encountered in the Baltic which suggests that this factor determines its absence from that sea. The lower limit which Calanus can tolerate in the laboratory is about 17‰, a value lower than is found in the open ocean.

Since Calanus can always escape from the injurious effect of light by going deeper into the water, this is not a factor which will affect its geographical distribution, but it is possibly one of the causes of its vertical distribution and diurnal migration. Much work has already been done on these lines (Esterley, 1919 ; Russell, 1928) and it need here only be noted that there is a striking contradiction between the fact that Calanus is

injured by strong light and that at times it swarms at the surface in bright sunlight.

Food Requirements of Calanus.

Respiration gives a measure of food requirements since there is a quantitative relation between the amount of oxygen used and the amount of material combusted to produce energy. The lack of figures for the carbon dioxide exchange makes it impossible to state the nature of the material utilised.

Ostenfeld (1913) estimated the amount of oxygen used by adult *Calanus hyperboreus* and found it amounted to 0·68 ml. per 1000 per hour which, since *C. hyperboreus* is larger than *C. finmarchicus*, agrees very well with our figures. Pütter made a long series of experiments on the respiration of copepods (1922, 1923, 1924–25), but apparently only a few were done on Calanus. The majority were on mixed catches of small copepods (Pseudocalanus, Centropages, Paracalanus, Acartia and Oithona) and the results varied a good deal from one experiment to another. In general he found that respiration is considerably higher in summer than in winter (at the same temperature), and that copepods require as food from 39% (for Calanus) to 156% (for Oithona) of their body weight daily in summer (1924–25). This involves a very large intake of food. His calculations of the enormous number of diatoms or other organisms necessary are well known. His experimental methods and the assumptions on which his calculations are based have been criticised by Krogh (1931) and from our results it certainly appears that his values for respiration and maintenance are excessive.

For Calanus, at 17·7° C., he records a utilisation of 1·83 ml. per 1000 per hour (1922) or roughly three times our value in the dark. From January to March the Stage V weigh about 15 mg. per 100 individuals (Marshall, Nicholls and Orr, 1934) and the oxygen consumption at 5° C. is about 0·17 ml. per 1000 per hour (Figure 2 and Table II). From April to July they weigh on an average about 30 mg. per 100 individuals and the oxygen consumption is about 0·42 ml. per 1000 per hour at 15° C. From these figures it follows that the food requirements of Stage V Calanus in winter lie between 0·002 and 0·006 mg. per individual per day, and in summer between 0·005 and 0·013 mg. The lower value given is that for fat and the higher that for carbohydrate in each case. The real value lies between these and depends on the composition of the food utilised. These figures indicate that for Stage V (the most abundant form) the amount of food required daily, expressed as a percentage of the body weight (dry) of one Calanus, lies between 1·3 and 3·6 in winter, and 1·7 and 4·5 in summer. For adults the percentage lies between 2·2 and 2·8 in winter, and 6·2 and 7·6 in summer. These values are very much lower than that calculated by Pütter for Calanus (38·7%).

A comparison of the food requirements with the food available as microplankton could be made from our knowledge of the seasonal fluctuations of the latter and the analyses of Brandt (1898) and Brandt and Raben (1919–22). In view, however, of our very incomplete knowledge of the composition of the different organisms used by Calanus and our ignorance of the digestibility of these by Calanus, any attempt at a direct relation should be deferred till further work has been done.

SUMMARY.

1. Experiments have been done to determine the oxygen utilisation by male, female and Stage V Calanus under different environmental conditions.

2. An initial fall in the respiration of adult Calanus was observed during the first few hours after capture. Stage V do not show this clearly. It is found more often in winter than in summer.

3. The lethal temperature varies from 24° C. in winter to 26° C. in summer. Stage V Calanus are more resistant to high temperatures than adults.

4. Respiration rises with increase of temperature from 0° C. to 20° C The increase does not follow van't Hoff's law. The oxygen consumption of males and females is about the same, while that of Stage V is lower. Above 20° C. there is a harmful effect.

5. Within the limits studied (pH 7·4–pH 8·5) change in hydrogen-ion concentration has no effect on respiration.

6. Calanus are unaffected by an increase in the oxygen content of the water, but are sensitive to low oxygen tensions. Below a concentration of about 3 ml. per litre the respiration decreases. At concentrations between 1 and 2 ml. per litre they are killed. They are more resistant at 5° C. than at 15° C. and Stage V are more resistant than adults at both these temperatures.

7. Calanus can become acclimatised to salinities as low as 35–40% seawater (S=12‰–13·6‰), but their respiration is lowered at a salinity of 50%.

8. Light has a striking effect on Calanus. It may increase the respiration by 100% or more. This effect can be detected also in the sea, but not below 5 metres. Continuous exposure to light is harmful.

9. The bearing of these results on the distribution of Calanus is discussed.

10. From the amount of oxygen used in respiration, calculations of the food required are made and these are compared with the results given by Pütter for Calanus and other copepods.

REFERENCES.

ANDERSON, W. T., and ROBINSON, F. W. 1925. An Oxalic Acid-Uranyl Sulphate Ultra-violet Radiometer. Journ. Amer. Chem. Soc., **47**, p. 718.

BEADLE, L. C. 1931. The Effect of Salinity Changes on the Water Content and Respiration of Marine Invertebrates. Journ. Exp. Biol., **8**, p. 211.

BĚLEHRÁDEK, J. 1930. Temperature coefficients in Biology. Biol. Rev., **5**, p. 30.

BRANDT, K. 1898. Beiträge zur Kenntniss der chemischen Zusammensetzung des Planktons. Wiss. Meeresunters., **3**, Abt. Kiel, p. 43.

BRANDT, K., and RABEN, E. 1919–1922. Zur Kenntniss der chemischen Zusammensetzung des Planktons und einiger Bodenorganismen. Wiss. Meeresunters., N.F., **19**, Abt. Kiel, p. 175.

BROWN, L. A. 1929. The Natural History of Cladocerans in relation to Temperature. I. Distribution and Temperature Limits for vital activities. Amer. Nat., **63** (686), p. 248.

EGE, R., and KROGH, A. 1915–16. On the relation between the temperature and the respiratory exchange in fishes. Internat. Rev. d. ges. Hydrobiol. u. Hydrograph., **7**, p. 48.

ESTERLEY, C. O. 1919. Reactions of various Plankton Animals with Reference to their Diurnal Migrations. Univ. Calif. Publ. Zool., **19**, p. 1.

FARRAN, G. P. 1911. Copepoda. Cons. Perm. Internat. p. l'explor. d. l. mer. Bull. trim., Part 2, p. 81.

HARVEY, J. M. 1930. The Action of Light on *Calanus finmarchicus* (Gunner) as determined by its effect on the Heart rate. Contrib. Can. Biol., **5**, p. 83.

HENZE, M. 1910. Uber den Einfluss des Sauerstoffdrucks auf den Gaswechsel einiger Meerestiere. Biochem. Zeitschr., **26**, p. 255.

HUNTSMAN, A. G. 1925. Limiting Factors for Marine Animals. I. The lethal effect of Sunlight. Contrib. Can. Biol., **2**, p. 81.

HUNTSMAN, A. G., and SPARKS, M. L. 1925. Limiting Factors for Marine Animals. III. Relative resistance to high temperatures. Contrib. Can. Biol., **2**, p. 95.

HYMAN, L. H. 1930. The effect of Oxygen Tension on Oxygen consumption in Planaria and some Echinoderms. Physiol. Zool., **2** (4), p. 505.

KEYS, A. B. 1930. The Measurement of the Respiratory Exchange of Aquatic Animals. Biol. Bull., **59**, p. 187.

KLUGH, A. B. 1929. The Effect of the U.V. Component of Sunlight on certain Marine Organisms. Canad. J. Research., **1** (1), p. 100.

—— 1930. The Effect of the U.V. Component of Sunlight on certain aquatic Organisms. Ibid., **3** (2), p. 104.

KREPS, E. 1929. Untersuchungen über den respiratorischen Gaswechsel bei *Balanus crenatus* bei verschiedenem Salzgehalt des Aussenmilieus. I. Mitt. Über den Sauerstoffverbrauch im Wassermilieu bei verschiedenem Salzgehalt. Pflüger's Arch. f. d. ges. Physiol., **222**, p. 215.

KROGH, A. 1931. Dissolved substances as Food of Aquatic Organisms. Biol. Rev., **6**, p. 412.

LEPESCHKIN, W. W. 1931. Some Experiments on the Influence of Light and Poisons on Marine Copepods, with reference to their Daily Migration. Bull. Scripps Inst. Oceanog., **3**, p. 33.

MARSHALL, S. M., NICHOLLS, A. G., and ORR, A. P. 1934. On the Biology of *Calanus finmarchicus*. V. Seasonal Distribution, Size, Weight and Chemical Composition in Loch Striven in 1933, and their relation to the Phytoplankton. Journ. Mar. Biol. Assoc., **20**, p. 793.

MARSHALL, S. M., and ORR, A. P. 1927. The Relation of the Plankton to some Chemical and Physical Factors in the Clyde Sea Area. *Idem*, **14**, p. 837.

NIKITIN, W. N. 1931. Die untere Planktongrenze und deren Verteilung im Schwarzen Meer. Internat. Rev. d. ges. Hydrobiol. u. Hydrograph., **25**, p. 102.

ORR, A. P. 1934. On the Biology of *Calanus finmarchicus*. IV. Seasonal Changes in the Weight and Chemical Composition of Calanus from Loch Fyne. Journ. Mar. Biol. Assoc., **20**, p. 613.

OSTENFELD, C. H. 1913. De danske Farvandes Plankton i Aarene 1898–1901. III. Phytoplankton og Protozoer (avec un résumé en français). K. Danske Vidensk. selsk. Skr. Raekke 7 Naturvid. Afd., **9**, p. 113.

POWERS, E. B. 1930. The Relation between pH and Aquatic Animals. Amer. Nat., **64** (693), p. 342.

PÜTTER, A. 1909. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Jena 1909, pp. 1–168.

—— 1922. Die Frage der parenteralen Ernährung der Wassertiere. Biol. Centralbl., **42**, p. 72.

—— 1923. Der Stoffwechsel der Copepoden (zugleich ein Beispiel für die Verwendung der Korrelationsmethode in der Physiologie). Pflüger's Arch. f. d. g. Physiol., **201**, p. 503.

—— 1924–25. Die Ernährung der Copepoden. Arch. für Hydrobiol., **15**, p. 70.

RUSSELL, F. S. 1928. The Vertical Distribution of Marine Macroplankton. VII. Observations on the Behaviour of *Calanus finmarchicus*. Journ. Mar. Biol. Assoc., **15**, p. 429.

The Larval Stages of *Longipedia coronata* Claus, *L. scotti* G. O. Sars, and *L. minor* T. and A. Scott, with a Description of the Male of *L. scotti*.

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With 8 Figures in the Text.

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Introduction.

During the course of some routine plankton work over the years 1931-33, embracing collections from various parts of the Clyde sea-area, nauplii of a peculiar appearance were noticed and many were removed for further examination. In this way over 300 specimens were secured, which on measurement showed five clear size groups, corresponding to five different stages. This nauplius was identified as one of the Longipediidæ (Copepoda), described and illustrated by Gurney (1930, p. 469) as "Genus III." Hauls in Loch Striven in 1934 showed large numbers of this nauplius

early in April, and many were isolated alive in an endeavour to rear them and so trace their origin. As pointed out by Gurney they are very easily caught on the surface film, which makes them difficult to rear in open vessels. By placing them under a system of water circulation from which all air was excluded it has been possible to rear them to the first copepodite stage. The later copepodites are easily reared in small open dishes containing a little mud, covered with about 10 c.c. of water. Nauplii

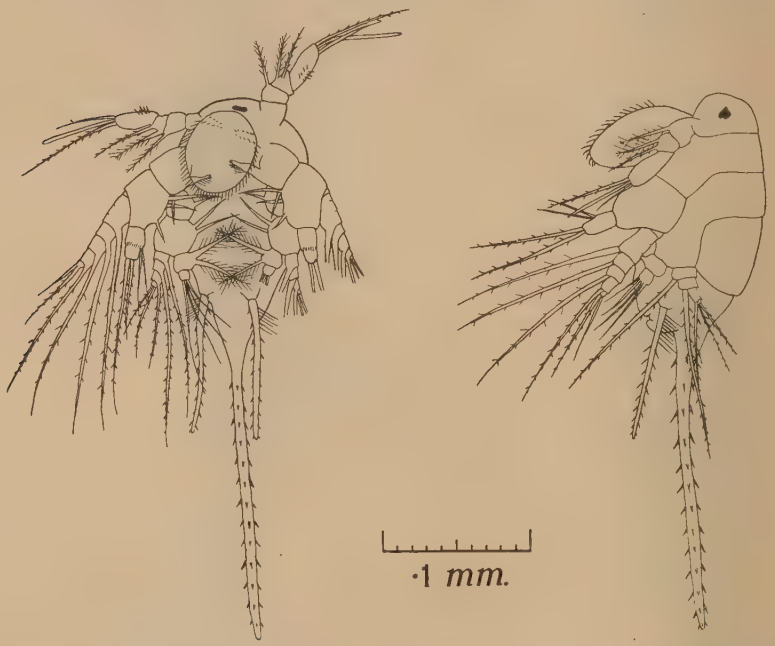

Fig. 1.—The first nauplius of *L. coronata* from below and from the left side. The specimen illustrated was peculiar in showing the three primary segments of the head region, corresponding to the first three nauplius appendages. No other specimen was seen showing this structure which must not be regarded as normal for the first nauplius of this species.

have also been successfully reared in open glass tubes, $6'' \times 1''$, which provide a sufficient depth of water to reduce the chances of their becoming caught in the surface film. At the second copepodite stage the animal is definitely recognisable as a Longipedia by the elongated endopod of the second leg (see also Gurney, 1930, p. 467). The larva has been reared through all its stages of development and identified as that of *Longipedia coronata*.

Ovigerous females of this species were obtained by using a bottom net ; and on hatching the eggs, the first nauplius, not previously seen in plankton

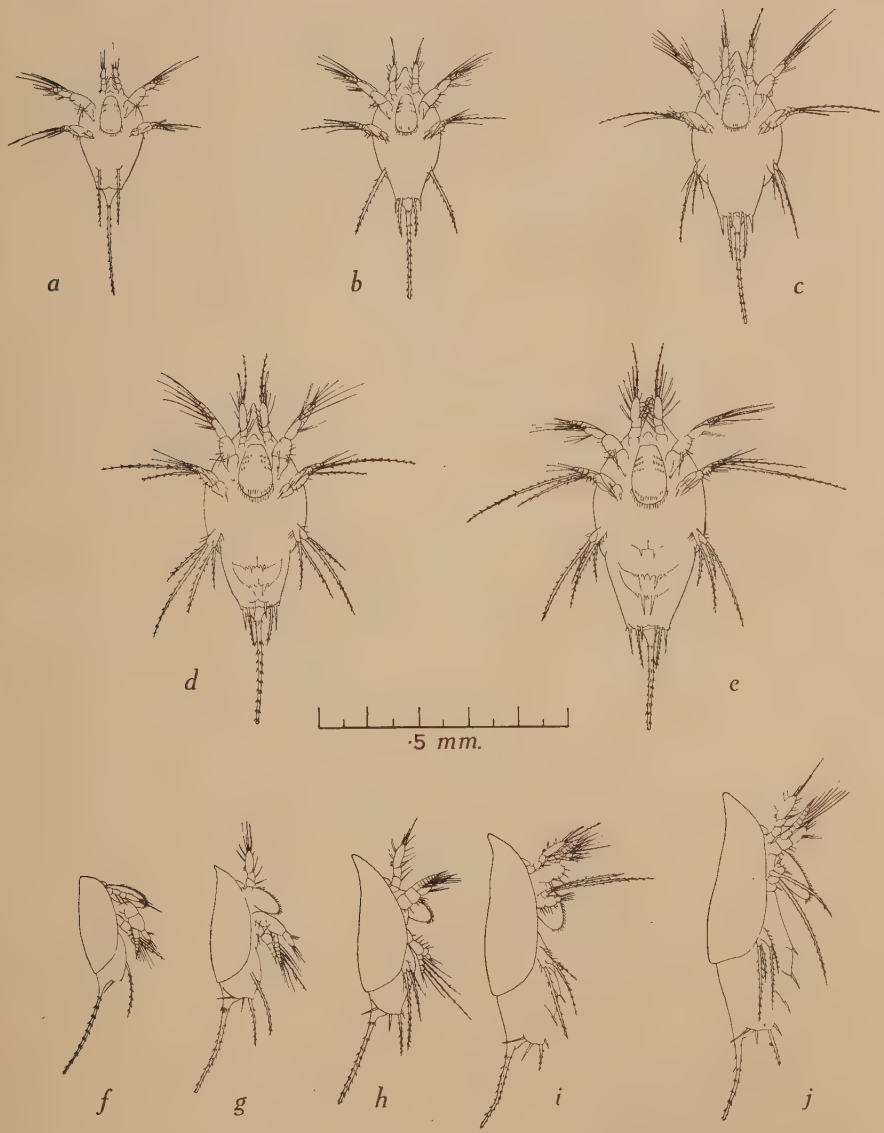

FIG. 2.—The second to sixth nauplii of *L. coronata*; *a* to *e*, in ventral view; *f* to *j*, in lateral view.

samples, has been found. This has proved interesting in that it resembles in shape the Longipedia nauplius described by Gurney (1930, p. 463) as the " green form." There is no anterior projection of the head region and this seems to be typical of the genus, modification in shape occurring only in later stages.

In the search for the adult of *L. coronata*, specimens of *L. scotti* (not previously recorded from this area) were obtained and these, when their eggs were hatched, proved to have a first nauplius indistinguishable, even in size, from that of *L. coronata*. In the later nauplii, however, the posterior terminal spine shortens and the larva assumes the form of that described by Gurney (1930, p. 464, Fig. 2) as the " pink form." All the

Fig. 3.—The antennules of nauplii two to six of *L. coronata*.

nauplius stages of this form also have been reared and since it is less liable to be held in the surface film its rearing does not present the same difficulty.

The nauplii of *L. coronata* are illustrated in full. The structure of the appendages differs in only minor details from that of the " green form " described by Gurney.

The Nauplii of *L. coronata*.

The nauplius was first described and figured by Gurney (1930) and is peculiar " in the long narrow shape of the body and remarkable helmet-shaped head. Not only is the head produced forwards and upwards but it has also a downward beak-like projection " (p. 469). These features can be seen in Figure 2 and the very prominent labrum, covered with small spines and fine hairs, will be noticed. An eye is visible in fresh material.

The Antennule (Figs. 1 and 3).

This has four segments, the first being unarmed ; the second bears one plumose seta ; the third, one plumose and one spinous seta. The armature of these three segments is constant throughout the six nauplii. The terminal segment is elongated and bears one spinous seta and an æsthetasc terminally, with a number of subsidiary terminal and lateral setæ varying from one in the first nauplius to fourteen in the sixth. A double row of short bristles is present on the outer edge of this segment, the number increasing from first to last nauplius. The changes that take place in this appendage are seen in Table I.

TABLE I.

SEGMENTATION AND ARMATURE OF ANTENNULE.

" A " indicates Æsthetasc.

Segment.	Nauplius Stage					
	I	II	III	IV	V	VI
1	–	–	–	–	–	–
2	1	1	1	1	1	1
3	2	2	2	2	2	2
4	2+A	3+A	6+A	10+A	13+A	15+A

The Antenna (Figs. 1 and 4).

The coxopodite and basipodite are armed with strong masticatory spines and setæ ; the unsegmented endopodite has rigid spinous terminal setæ and a group of lateral setæ on the inner edge ; the exopodite is long and well developed with many segments, five in the first nauplius, increasing by one segment with each moult to the maximum of eight in the fourth, fifth and sixth nauplii (Table II).

TABLE II.

SEGMENTATION AND ARMATURE OF THE ANTENNA.

Stage.	Coxopodite.	Basipodite.	Endopodite.	Exopodite Segments							
				1	2	3	4	5	6	7	8
I	1+1	3	2+2			1		1	1	1	2
II	1+1	3	2+3		1		1	1	1	1	2
III	1+1	4	3+4	1		1	1	1	1	1	3
IV	2+1	5	3+4	1	1	1	1	1	1	1	3
V	2+1	5	4+5	2	1	1	1	1	1	1	3
VI	2+1	5	4+5	2	1	1	1	1	1	1	3

There is no further development of the number of setæ from the fifth to the sixth nauplius but the large masticatory spine on the coxopodite is considerably enlarged at this moult.

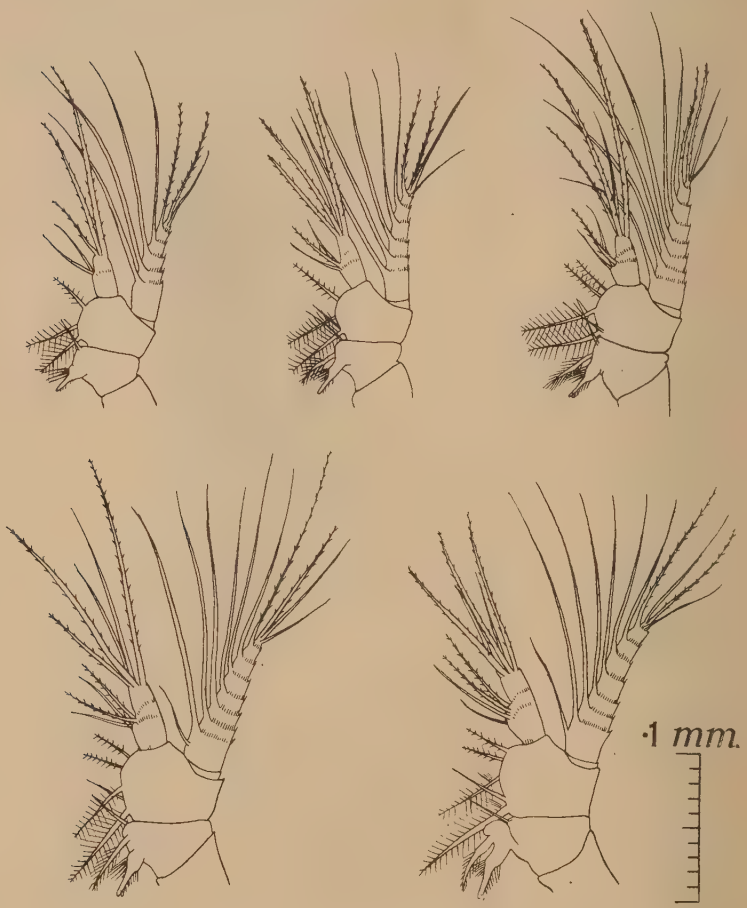

Fig. 4.—The antennæ of nauplii two to six of *L. coronata*.

The Mandible (Figs. 1 and 5).

There is a very small coxopodite bearing a single plumose seta. The basipodite is more strongly armed with a number of plumose setæ but the main armature of this masticatory appendage is the powerful spine on the enlarged first segment of the endopodite, which has in addition a number of plumose setæ. The terminal segment of the endopodite bears a group

of unplumed setæ. The exopodite is four-segmented in all stages and bears rigid spinous setæ, two of which are particularly long and strong. The changes in this appendage are summarised in Table III.

TABLE III.

SEGMENTATION AND ARMATURE OF THE MANDIBLE.

Stage.	Coxopodite.	Basipodite.	Endopodite		Exopodite.			
			1	2	1	2	3	4
I	1	2	1+1	4	1	1	1	2
II	1	3	3+1	5	2	1	1	2
III	1	4	3+1	6	2	1	1	2
IV	1	7	4+1	6	2	1	1	2
V	1	7	4+1	6	2	1	1	2
VI	1	8	4+1	6	2	1	1	2

The Maxillule (Figs. 2 and 6).

This appendage is represented in the first three nauplii by a single large spine, thickly covered with small spines and in the first nauplius bearing a number of fine hairs at the base. These hairs are lacking in the second and third nauplii. In subsequent stages a two-segmented appendage is present bearing large terminal spines and small lateral setæ (Table IV).

TABLE IV.

SEGMENTATION AND ARMATURE OF THE MAXILLULE

Segment	Nauplius Stage					
	I	II	III	IV	V	VI
Endopodite	Large Spine			2+3	2+6	2+6
Exopodite				2+1	2+3	2+3

Other Appendages (Fig. 2).

The first two pairs of swimming feet present in the first copepodite are represented in the fifth and sixth nauplii as " ridges with spines, but are not definitely bilobed " (Gurney, 1930, p. 466). The maxillæ and maxillipeds are situated just behind the maxillules but are not clearly separable in *L. coronata*.

The Caudal Region.

A pair of short, backwardly directed setæ first appear in the second nauplius in the region of the anus. To these are added in the third nauplius one pair of short setæ, upwardly directed and situated dorsal to the first pair, and a second pair of long, spinous setæ postero-ventrally (Fig. 2, *f–j*). In the fourth nauplius one pair of short spines appears just

anterior to the postero-ventral pair. The single median posterior spine, most prominent of all, is present in all stages, and characteristic of the nauplii of the genus. The arrangement of spinules upon this spine appears to be spiral.

The Nauplii of *L. scotti*.

The " pink form " of Gurney undoubtedly belongs to this species and nauplii reared from females with egg-sacs have been carried through to

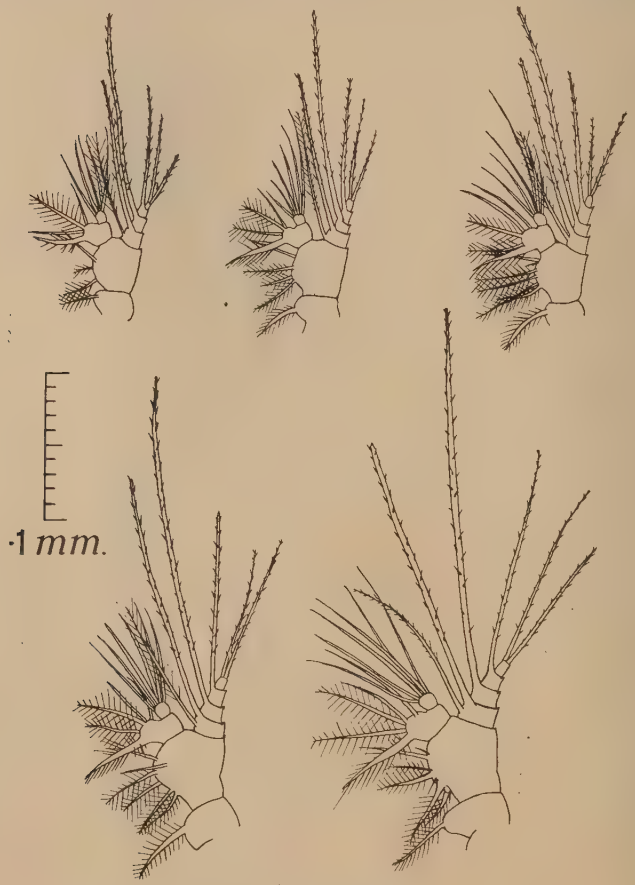

Fig. 5.—The mandibles of nauplii two to six of *L. coronata*.

the fifth copepodite, while later nauplii collected from the plankton have been reared to the adult stage. The male has thus been obtained and will be described later (p. 43).

Again six nauplius stages are present, the first indistinguishable from that of *L. coronata*, the later stages showing the modification of shape peculiar to this species—namely, the broad, rounded anterior; the square labrum; the shortening of the posterior spine; and the presence of a tooth on the basipodite of the antenna. The patchy coloration which develops in the later nauplii is also distinctive.

Apart from these features, mentioned by Gurney (1930, p. 467) the exopodite of the maxillule shows a structure different from that of the preceding species. The exopod in *L. coronata* bears one rigid spine and a

Fig. 6.—The maxillules of nauplii four to six of *L. coronata*.

number of shorter setæ, whereas in *L. scotti* the rigid spine is replaced by a group of setæ of sub-equal length (see Gurney, 1930, Fig. 2). The tooth on the basipodite of the antenna first appears in the second nauplius. For the rest, the appendages resemble those figured and described for *L. coronata*.

The Nauplii of *L. minor*.

One specimen of a small yellow nauplius in the fifth stage was obtained from the plankton. This moulted through successfully to become an adult female, quite clearly *L. minor*. An examination of the moults of this nauplius showed that it was identical in structure with the "green form" of Gurney. The apparent discrepancy here with Gurney's colour description is, I think, easily explained. The depth of pigmentation varies in different individual nauplii of a species, and when the natural coloration is absent or greatly reduced, the yellow colour of the food mass predominates, leading one to ascribe that colour to the nauplius as a whole in the absence of any other. Nauplii of both *L. coronata* and *L. scotti* have been obtained in which the usual red or pink colour has been absent, and

the only colour noticeable has been the greenish yellow of the gut contents. Gurney described the green form and stated that the yellow form was " very rare by comparison." It so happens that the single specimen of this species obtained here lacked the green pigmentation and so appeared to be yellow.

The maxillule in this nauplius is of the *L. scotti* type. A point of interest is that in the sixth nauplius of this species the maxilla and maxilliped are much more clearly separated than in either of the preceding species. One further point should be noticed, namely that there are four segments in the antennule in all three species, the proximal one being unarmed throughout.

Average Lengths of the Nauplii in mm.

Nauplius Stage.	*L. coronata.*				*L. scotti.*				*L. minor.**			
	Body	Spine	Total	S/T	Body	Spine	Total	S/T	Body	Spine	Total	S/T
I	·15	·19	·34	·6	·15	·15	·30	·5	·13	·14	·27	·5
II	·20	·22	·42	·5	·19	·11	·30	·4	·18	·17	·35	·5
III	·26	·21	·47	·4	·24	·06	·30	·2	·19	·18	·37	·5
IV	·33	·20	·53	·4	·27	·07	·34	·2	·22	·19	·41	·5
V	·40	·16	·56	·3	·32	·10	·42	·2	·27	·18	·45	·4
VI	·45	·21	·66	·3	·37	·08	·45	·2	·30	·18	·48	·4

This table gives the lengths of the body and of the spine, and the ratio spine to total length (S/T), for all stages of the three species under discussion. It is interesting to see that in *L. coronata*, while the body shows a steady increase in length, the spine remains more or less constant and, therefore, grows relatively shorter. In *L. scotti* while the body grows in length the spine shortens considerably, giving a marked relative decrease. In *L. minor* the spine shows an increase in length as the body grows, thus showing very little relative shortening.

The Copepodite Stages of Longipedia.

The early copepodite stages of Longipedia, showing the development of the second leg, have been described and illustrated by Gurney (1930), and it will suffice here to give a summary in tabular form of the number of segments and swimming legs present in each stage. In the table below, a segment bearing no appendages is indicated by " × " ; " r " indicates that the limb is rudimentary ; " f " stands for the caudal furcæ.

Though some authors (Claus, 1863 ; Sars, 1911 ; Wilson, 1932) have stated that the long endopod of the second leg may be used in locomotion, and though it might reasonably appear to be useful on muddy grounds such as Longipedia inhabits, yet during four months constant observation

* Lengths of the first four stages are taken from Gurney (1930) ; those of the fifth and sixth nauplii are lengths of moults of the single specimen obtained here and agree exactly with Gurney's measurements for these stages.

of the living animal, kept on mud, I have never seen the endopods used in the manner suggested by Sars. Normally the animal remains buried beneath the surface of the mud, through which it burrows vigorously, and if disturbed it swims actively about on its side or back, the endopods of the second legs taking no part in the swimming but remaining folded back along the body.

Copepodite Stage.	Cephalosome	Segments of Body. 2	3	4	5	6	7	8	9	10
I	p1	p2	p3r	×	×f					
II	p1	p2	p3	p4r	×	×f				
III	p1	p2	p3	p4	p5r	×	×f			
IV♂	p1	p2	p3	p4	p5	p6r	×	×f		
♀						×				
V♂	p1	p2	p3	p4	p5	p6	×	×	×f	
♀						×				
VI♂	p1	p2	p3	p4	p5	p6	×	×	×	×f
♀						×				

A Comparison with the Cirripedes.

Gurney (1931, p. 80) points out that Hansen in 1899 described the nauplius of Longipedia as that of an unknown copepod. He in turn refers to the earlier work of Claus (1863) who also recognised this larva as that of a copepod. All of these workers have remarked on the resemblance between this nauplius and that of the cirripede, and the relationship of the family Longipediidæ has been fully discussed by Gurney (1930, p. 472). There are one or two points of interest that have arisen which seem to stress the possible relation between Longipedia and the cirripedes.

While the general shape of the body of the nauplius seen from the dorsal view resembles that of a Calanoid (Gurney, 1930, p. 472), yet the later nauplii of *L. scotti* bear a striking resemblance to that of a cirripede when viewed from the side (see Fig. 7, *d* and *e*). A comparison of the appendages in Figures 3–5 with those of the cirripede (Groom, 1894) brings out other points of similarity in addition to those referred to by Gurney (1930), in particular the structure of the masticatory spine on the coxopodite of the antenna. Moreover the nauplii of Longipedia with four segments in the antennule occupy a position intermediate between those of the majority of copepods with three segments and those of the cirripedes with five.

Another point of considerable interest is the resemblance in the mode of swimming. Calanoid nauplii have a smooth, apparently effortless type of progression, whereas cirripede nauplii move in a laboured, jerky manner, performing circles on their backs. This exactly describes the swimming of

Longipedia nauplii. It may, of course, be only a mechanical effect due to the long posterior spine present in both. Harpacticoid nauplii are not normally pelagic and so cannot be compared, but if this apparent relation to the cirripedes is real it would strengthen the suggestion made by

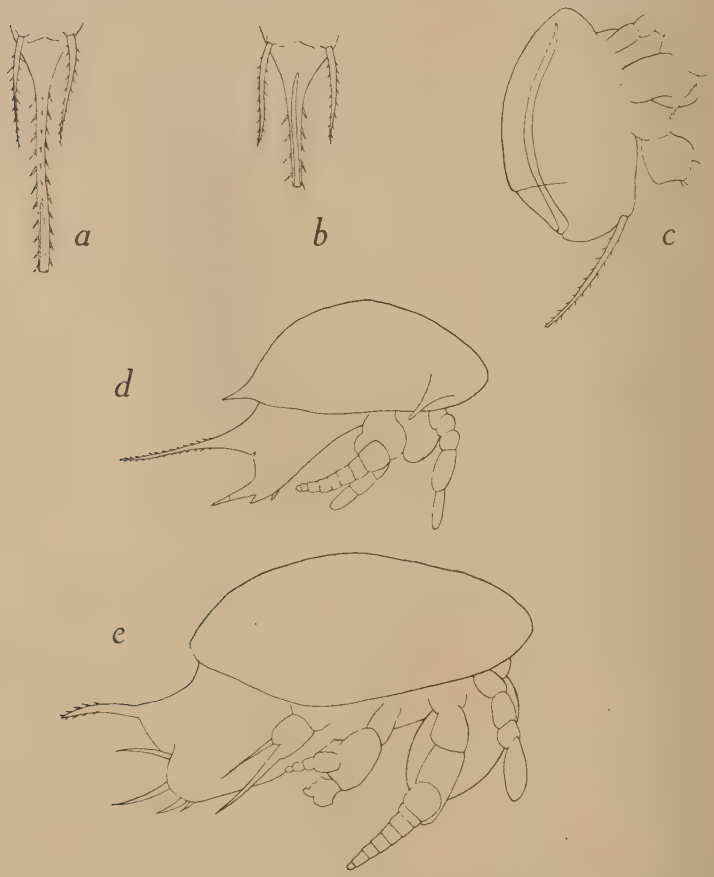

FIG. 7.—*a*, *b* and *c*, different stages of invagination of the posterior median spine of Longipedia nauplii. In *c* it is totally within the body. *d* and *e* are nauplii of a cirripede from the plankton and *L. scotti* respectively, drawn from the side to show similarity in shape.

Gurney (1930), from other considerations, that the pelagic state of the nauplii of Longipedia is primitive.

A minor point of similarity is that in the first nauplius of the cirripede *Lepas anatifera* (Groom, 1894, p. 167, Fig. 153a) the caudal spine is invaginated within the body. Numerous individuals of Longipedia

nauplii have hatched with the spine partly invaginated (Fig. 7, *a* and *b*) and one whole batch of *L. scotti* nauplii hatched apparently without tail spines. Closer examination showed that the spines were entirely invaginated within the bodies of the nauplii (Fig. 7, *c*). This is apparently the normal condition in the developing eggs and in this case the spines had failed to be evaginated at the time of hatching. None of this brood of nauplii lived to become second nauplii. This state of invagination in spines during development is not, however, restricted to these two groups.

Breeding and Development.

It appears that having once received a spermatophore the female produces consecutive broods of eggs for the rest of the breeding season. Active breeding had certainly begun by the beginning of April (large numbers of nauplii and first and second copepodites were taken in the plankton on 6.4.34) and continued (in the laboratory) until the beginning of August. Nauplii were taken occasionally earlier in the year from Keppel Pier and the routine hauls in Loch Striven in 1933 showed them to be present in January. No breeding females lived beyond the beginning of August, but before death one specimen of *L. scotti* had produced at least nine batches of eggs. (It is, of course, impossible to say how many broods it had produced before capture.) An egg-sac was present when the specimen was caught on April 15th ; the nauplii hatched within two days and four more broods had appeared by the beginning of June, at intervals of five or six days and taking on the average as many days to develop. After a rest of 15 days breeding began again and three more broods appeared. A further rest of 10 days was followed by the production of the ninth egg-sac, the nauplii taking six days to hatch. A few days later this female died an apparently natural death.

About 14–20 nauplii hatched from each egg-sac in the early part of the year, but of the fourth and fifth broods only one hatched from each, and it appeared that the supply of spermatozoa might have been exhausted. The sixth egg-sac, however, produced 11 nauplii and the seventh two or three, but no nauplii were seen from either of the last two broods.

A specimen of *L. coronata* produced seven batches of eggs between April 18th and July 4th after which it died. In the case of this specimen there was clear evidence of a speeding up of reproduction as the summer advanced and the (laboratory) temperature rose. The first three broods took on the average nine days to develop with four-day intervals. A thirteen-day interval followed and then a fourth brood appeared. The remaining three broods took an average of six days to develop, with one-day intervals.

There is no evidence of parthenogenesis in Longipedia such as has been

recorded in Canthocamptus (Gurney, 1932, p. 109). A specimen of *L. coronata* reared from the second copepodite lived for 44 days as an adult female, with one ovary developed, but no eggs were spawned ; similarly the specimen of *L. minor* reared from the fifth nauplius lived as an adult female for a similar period with well developed ovaries, but did not spawn.

Three nauplii of *L. coronata* hatched on June 8th became first copepodites on 21st and 22nd of that month, having taken 13–14 days and one of a batch of *L. scotti* nauplii, hatched on May 28th, became a first copepodite on June 14th, taking 17 days. The time taken in development varies, of course, with temperature and abundance of food and these figures, therefore, cannot be regarded as more than an indication of the normal time. At first, copepodites were kept on muddy sand similar to that of their natural habitat ; no food was added and development proceeded slowly. Later, diatoms were added and the rate increased. Three different specimens took 27, 21 and 33 days to develop from first to sixth copepodites (May to July).

Feeding in Longipedia.

An adult female of *L. scotti* and a specimen of *L. minor* while a third copepodite, were observed feeding on diatoms. The process was the same in both cases. The mouth parts normally are in a state of constant vibration, the antennules and antennæ not taking part in the vibration. The animal feeds while lying on its side or back and the current caused by the vibration of the mouth parts brings diatoms, detritus, etc., directly towards the mouth from the sides and front of the animal. The outgoing current passes ventrally along the body between the swimming legs. Diatom chains, and Phæocystis colonies were observed drifting towards the mouth, the former being held by the antennæ and passed in end on. Phæocystis colonies were broken up by the mandibles and as much as possible ingested. A large number of cells was lost and a fine stream of these was observed passing away in the ventral current. Colonies were dealt with one at a time and if a second arrived before one had been broken up, this was held away from the mouth parts by the antennæ and passed on at the right moment. The anterior portion of the gut was thus gradually filled and a fæcal pellet, which meanwhile had been formed from the contents of the posterior portion, was then forcibly expelled. All vibration ceased during this process. Vigorous peristalsis refilled the posterior portion of the gut and feeding proceeded. In a steadily feeding animal pellets were expelled about once an hour and food passed through in about two hours (demonstrated by inducing a specimen to accept some carmine granules). Longipedia when feeding is partially selective. A pellet recently expelled was presented for ingestion, but though broken

up by the mouth parts none of it was ingested. The carmine was ingested in small quantities with other food.

The Male of *Longipedia scotti*.

Though four species of Longipedia have been distinguished by Sars in his excellent monograph on the Crustacea of Norway (1911), the male

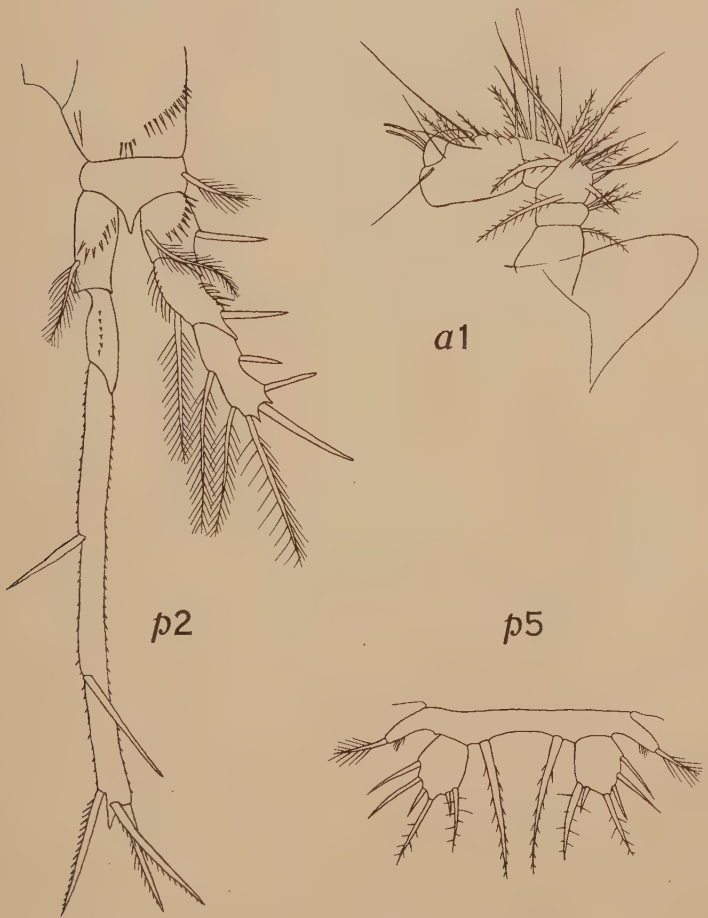

Fig. 8.—The antennule, second and fifth legs of the male of *L. scotti*.

of only one was included, that of the type species, *L. coronata*. That of *L. minor* had already been described by T. Scott (1893, p. 200). So far as I am aware the male has not since been described for either of the two

remaining species. While rearing specimens of this genus a number of males appeared belonging to both *L. coronata* and *L. scotti*, but only one of the latter became adult.

The male is easily recognisable as such in the fourth copepodite stage by the presence of "lappets" on the genital segment. The typical male prehensile antennule is not developed until the adult state is attained.

The male of *L. scotti* is slightly smaller than the female and can be distinguished by the structure of the antennule; by the presence of only two lateral spines on the terminal segment of the endopod of the second leg, in which it differs from all the other forms of Longipedia described by Sars, but resembles the male of *L. minor* (see Scott, 1893); by the fifth legs which show slight differences from those of the female; and by the genital lappets already referred to. The last three segments of the urosome bear fringes of denticles similar to those of the female, but they are more clearly defined ventrally than dorsally. The anal operculum is identical with that of the female (see Sars, Pl. V).

The chief features of the male of this species are illustrated in Figure 8 from the adult of the single specimen which attained maturity in the laboratory. No males of this species and but a single male of *L. minor* have been taken from the sea, and this shows close agreement with Scott's drawings. Apart from its smaller size it is easily distinguished from the male of *L. scotti*, although both have only two spines on the long terminal segment of the endopod of the second leg, since it is the large external spine which is absent in both cases and the remaining two inner spines occupy the same position as in the respective females.

An interesting point is the presence of spinules on the setæ of the caudal furcæ in all the pre-adult stages of both sexes of all three species of Longipedia examined; but these are lost in the last moult. (Only the female of *L. minor* has been seen in the pre-adult stages.) Sars has shown that spinules on these setæ are present in the adult female of *L. rosea* and form one of the diagnostic characters of this species.

It may also be worth noting that the fringe of denticles on the posterior edge of the segments of the urosome is present not only in the adults of *L. scotti* but also in the pre-adult stages of the male of *L. coronata*.

All the drawings have been made with the aid of a camera lucida.

It is a pleasure to express here my thanks to Dr. R. Gurney for the interest he has shown in the paper and for reading the MS. before publication.

SUMMARY.

1. The nauplius stages of three species of Longipedia are described, and the segmentation and armature of the appendages of *L. coronata* are given in tabular form.

2. The main features in the external structure of the copepodite stages are summarised in a table.

3. Additional points of resemblance between the nauplii of Longipedia and the cirripedes are presented.

4. Notes are given on breeding and development under laboratory conditions ; there is no evidence of parthenogenesis.

5. Feeding in the copepodite stage of Longipedia is described.

6. A description is given of the hitherto unknown male form of *L. scotti*.

REFERENCES.

Claus, C. 1863. Die frei lebenden Copepoden. Leipzig.

Groom, T. T. 1894. On the Early Development of the Cirripedia. Phil. Trans. Roy. Soc., B, Vol. 185, pp. 119–232.

Gurney, R. 1930. The Larval Stages of the Copepod Longipedia. Journ. Mar. Biol. Assoc., N.S., Vol. XVI, pp. 461–474.

—— 1931. British Freshwater Copepoda. Vol. I. Ray Society, London.

—— 1932. *Idem*, Vol. II, *idem*.

Hansen, H. J. 1899. Die Cladoceren und Cirripeden der Plankton-Expedition. Ergeb. d. Plankt.-Exped. d. Humboldt-Stiftung. Bd. II.

Sars, G. O. 1911. An Account of the Crustacea of Norway. Vol. V., Copepoda (Harpacticoida). Bergen.

Scott, T. 1893. Additions to the Fauna of the Firth of Forth. Pt. V., 11th Ann. Rep. Fish. Board Scot. Pt. III.

Wilson, C. B. 1932. Copepods of the Woods Hole Region, Massachusetts. U.S. Nat. Mus., Bull. 158.

A List of British Invertebrates with their Characteristic Parasitic and Commensal Copepoda.

By

W. Harold Leigh-Sharpe, M.Sc.(Lond.).

Host.	Parasite.	Location.
	PORIFERA.	
Suberites domuncula Bowerbank. =*Ficulina ficus* (L.).	*Ascomyzon suberitis* (Giesbrecht) 1899.	Water passages.
Suberites spp.	*Ascomyzon latum* (Brady) 1880	Water passages.
	ECHINODERMATA.	
Echinus esculentus Linnæus.	*Ascomyzon latum* (Brady) 1880.	Surface.
	ANNULATA.	
Filograna implexa (Berkeley).	*Thaumaleus* [*Cymbasoma*] *filogranarum* (Malaquin) 1901.	Internal.
Gattyana cirrosa (Pallas).	*Hedyphanella superba* Leigh-Sharpe, 1926.	Dorsal surface.
Harmothoe impar (Johnston).	*Sarsilenium crassirostris* (M. Sars) 1870.	Dorsal surface.
Lagisca extenuata (Grube).	*Phallusiella psalliota* Leigh-Sharpe, 1926.	Dorsal surface.
Malmgrenia castanea McIntosh.	*Phallusiella vera* Leigh-Sharpe, 1926.	Dorsal surface.
Nereis spp.	*Nereicola ovata* Keferstein, 1863	Dorsal surface.
Polycirrus caliendrum Claparède.	*Saccopsis alleni* Brumpt, 1897.	Interior.
	CRUSTACEA.	
Homarus vulgaris Milne-Edwards. (=*Astacus homarus*.).	*Nicothoe astaci* Audouin and Milne-Edwards, 1826.	Gills.
Homarus vulgaris Milne-Edwards.	*Tisbe elongata* A. Scott, 1896.	Gills.
Paguridæ.	*Sunaristes paguri* Hesse.	In shells (? Commensal).
	MOLLUSCA.	
	(A) Lamellibranchiata.	
Cardium edule Linnæus.	*Paranthessius rostratus* (Canu) 1891.	Gonad.
Chlamys [*Æquipecten*] *opercularis* (Linnæus).	*Scottocheres elongatus* T. & A. Scott, 1894.	Mantle cavity.
,, ,,	*Paranthessius rostratus* (Canu) 1891.	Mantle cavity.
,, ,,	*Pseudanthessius thorelli* (Brady) 1880.	Mantle cavity.
,, ,,	*Modiolicola inermis* Canu, 1891.	Mantle cavity.

Host.	Parasite.	Location.
	MOLLUSCA.	
Ensis siliqua (Linnæus).	*Anthessius solenocurti* Della Valle, 1880.	Foot.
Paphia pullastra (Montagu).	*Paranthessius rostratus* (Canu) 1891.	Gonad.
Pecten maximus (Linnæus).	*Modiolicola inermis* Canu, 1891.	Mantle cavity.
	(B) Monotocardia.	
Buccinum undatum Linnæus.	*Anthessius arenicola* (Brady) 1872.	Surface.
	(C) Nudibranchia.	
Acanthodoris pilosa (Abilgaard).	*Splanchnotrophus gracilis* Hancock and Norman, 1863.	Surface of body.
Æolidia papillosa (Linnæus).	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
,, ,, ,,	*Splanchnotrophus angulatus* Hecht, 1893.	Body cavity.
Archidoris britannica (Johnston).	*Lichomolgus agilis* (Leydig) 1853.	Gills.
Eolidina glauca (Alder & Hancock).	*Splanchnotrophus angulatus* Hecht, 1893.	Body cavity.
Facelina longicornis (Montagu).	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
,, ,, ,,	*Splanchnotrophus willemi* Canu, 1891.	Body cavity and gonad.
Lomanotus genei Vérany.	*Splanchnotrophus insolens* T. & A. Scott, 1895.	Visceral cavity.
Idaliella aspersa (Alder & Hancock).	*Splanchnotrophus gracilis* Hancock & Norman, 1863.	Visceral cavity.
Idulia [*Doto*] *coronata* (Gmelin).	*Splanchnotrophus brevipes* Hancock & Norman, 1863.	Body cavity.
,, ,,	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
Idulia pinnatifida (Montagu).	*Splanchnotrophus brevipes* Hancock & Norman, 1863.	Body cavity.
Janolus cristatus (Della Chiaje).	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
Janolus hyalinus (Alder & Hancock).	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
Jorunna tomentosa (Cuvier).	*Lichomolgus agilis* (Leydig) 1853.	Papillæ.
Sphærostoma [*Tritonia*] *hombergi* Cuvier.	*Lichomolgus agilis* (Leydig) 1853.	Gills.
	(D) Cephalopoda.	
Benthoctopus ergasticus (P. & H. Fischer).	*Cholidya polypi* Farran, 1914.	Mantle cavity.
	UROCHORDA.	
Ascidia mentula Müller.	*Notopterophorus papilio* Hesse, 1864.	Branchial cavity.
Ascidiella aspersa (Müller).	*Ascidicola rosea* Thorell, 1859.	Branchial cavity.
,, ,, ,,	*Notodelphys allmani* Thorell, 1859.	Branchial cavity.
Botryllus schlosseri (Pallas).	*Botryllophilus ruber* Hesse, 1864.	Branchial cavity.
,, ,, ,,	*Enterocola fulgens* van Beneden, 1860.	Branchial cavity.
Phallusia mammillata (Cuvier).	*Notodelphys prasina* Thorell, 1859.	Branchial cavity.
Polycarpa sp.	*Doropygus pulex* Thorell, 1859.	Branchial chamber.

The Biology of *Balanus balanoides*. II. Algal Infection of the Shell.

By

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and

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With 2 Figures in the Text.

In work on the biology of *Balanus balanoides*, use has been made of the method of differentiating the successive year groups by the colour of their shells. The present paper describes the method of doing this, and gives a brief account of the infective penetrative algæ which cause the colouration. The first author (M.W.P.) is responsible for the algological observations, and the second (H.B.M.) for the zoological observations.

External Colour Changes.

On an exposed shore, devoid of fucoid vegetation, the barnacles frequently form in summer a broad band which has the appearance of having been whitewashed on the rocks. The same zone in winter is much less conspicuous, being greenish brown in colour. Closer inspection of the barnacles shows that in summer they have a greenish brown apex, and a basal zone of varying width which is pure white (Fig. 1). In the case of the first year spat, the entire shell is white at this season. In winter the entire shell is of a greenish brown colour, although the shade varies according to the age of the animal. Figure 2 is a diagrammatic representation of the typical colour changes in the barnacle shells at Port Erin, based partly on field observations, and partly also on sections of the barnacles. Briefly, the new shell formed in the summer (Fig. 2, *1*) gradually becomes coloured as the winter advances (Fig. 2, *2* and *3*), practically the whole shell being coloured by the following spring (Fig. 2, *4* and *5*). By early summer the old shell growth is sufficiently darkly pigmented to be sharply marked off from the new growth of white shell now making its appearance (Fig. 2, *6*). It will be noticed that the new area of white shell at this stage is disproportionately large, and the probable explanation of this is

discussed in the section on the abrasion of the shell. The same sequence of colour changes described for the first year, is followed in the second and third seasons by the newly formed shells of those years, while the old shell of previous years continues to darken slightly.

This series of changes was verified on animals of known age from patches of rock which had been scraped prior to spatfall, and thereafter contained barnacles of known age only. It may therefore be concluded

Photo H.B.M.

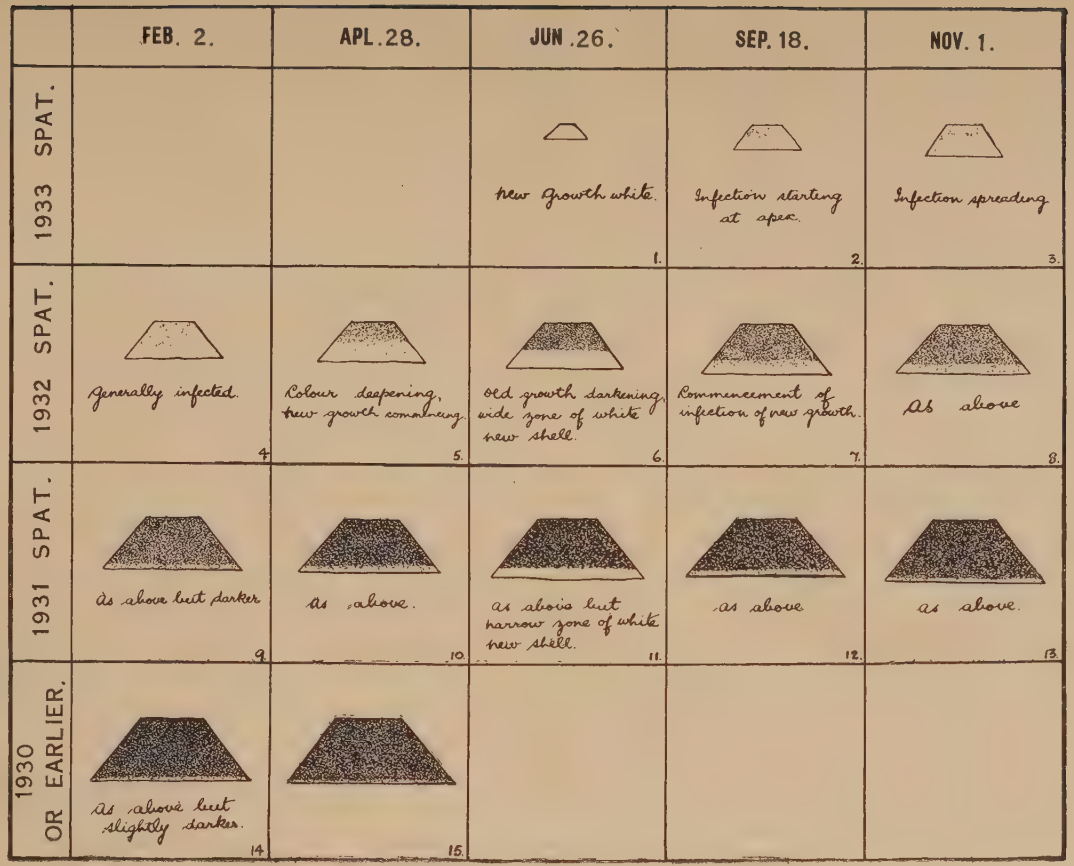
FIG. 1.—Barnacles of the 1932 and 1933 year groups, taken in Sept. 1933, showing algal infection of the upper zone of the older barnacles. × 2·5.

that, in this locality at least, colouration may be taken as a guide to the age of a barnacle up to two years old, and in some cases for a further year.

THE CAUSE OF COLOURATION.

Gruvel (1905) states that in some species of barnacles the shell is penetrated by algæ, of which he mentions the following: '*Hyella cæspitosa* (as *H. cæspitoda*), *Mastigocoleus testarum*, *Gomontia polyrhiza* and perhaps *Siphonocladus* sp. Cotton (1912) records *Plectonema terebrans* "in the shells of Balanus" from Clare Island, but states that it is rare.

In the present work, part of the identification of the infecting algæ, and the examination of their distribution in the shells, was carried out on fresh material from four Manx localities as follows: outside Bradda Head (very exposed to wave action), Port Erin Bay (fairly exposed), Alfred Pier, Port St. Mary (sheltered), and Inner Pier, Port St. Mary (very sheltered). The material was obtained from surface scrapings of the shells, as well as



M.W.P. DEL.

FIG. 2.—Schematic representation of seasonal colour changes in *Balanus balanoides* at Port Erin.

by complete decalcification of the barnacles, both methods yielding complete plants which were more readily identifiable than those seen in section. In addition sections were prepared from barnacles from various tidal levels, chiefly in Port Erin Bay, but to some extent also from outside Bradda Head. Collections were made in 1933 on February 12th, April 28th, June 26th, September 18th and November 1st, and in all 233 barnacles were sectioned. The material was fixed in Bouin's fluid, decalcified in 5% nitric acid in 70 % alcohol, cut at a thickness of 7μ and stained with Heidenhain's iron hæmatoxylin. The slides were originally prepared for examination of the condition of the gonads, but were found to be suitable also for showing the depth of algal penetration, and the approximate time of infection of the new shell zones.

The following is a list of all the species of algæ found by us penetrating the shells of the barnacles, together with notes on their distribution and fruiting.*

CHLOROPHYCEÆ.

Gomontia polyrhiza Born. et Flah. Figured, Newton, 1931, p. 96.

Perennial; from H.W.O.S.T.† to L.W.O.N.T.; generally distributed and very common; this species in particular is responsible for the green colouration of the shells; reproduction—sporangia present from December to March.

Ostreobium Queketti Born. et Flah. Figured, Newton 1931, p. 101.

Sporadic; from H.W.O.S.T. to L.W.O.N.T.; occasionally, outside Bradda Head; not in sufficient quantities to cause appreciable colouration.

CYANOPHYCEÆ.

Hyella cæspitosa Born. et Flah. Figured, Newton 1931, p. 13.

Perennial; from H.W.O.S.T. to L.W.O.N.T.; very common and generally distributed; chiefly responsible for the yellowish-brown colouration of the shells; reproduction—gonidangia present in winter and spring.

Plectonema terebrans Born. et Flah. Figured, Tilden 1910, Pl. XI, Fig. 6.

Perennial; from H.W.O.S.T. to L.W.O.N.T.; common and generally distributed; chiefly responsible for the mauve-green colouration of the shells.

Mastigocoleus testarum Lagerh. Figured, Newton 1931, p. 41.

* In addition, the following species were found growing on the outsides of the shells—*Fucus* spp. young plants, *Pelvetia canaliculata* Dene et Thur., *Ralfsia verrucosa* Aresch, forming brown patches on the shell surface, *Enteromorpha* spp. young plants, *Laurencia hybrida* Lenor., and the lichen *Lichina pygmæa* Ag.

† H.W.=high water, L.W.=low water, O.=ordinary, E.=equinoctial, S.T.=spring tide, N.T.=neap tide, M.S.L.=mean sea level.

Sporadic; L.W.O.N.T.; occasionally outside Bradda Head; not in sufficient quantities to colour the shell.

Microchæte grisea Thur. Figured, Newton 1931, p. 43.

Perennial; H.W.O.S.T. to L.W.O.N.T.; frequent and generally distributed; this species is generally recorded as growing on the surface of the shell, but here it appears to penetrate into it; not present in sufficient quantities to colour the shell; reproduction—at all times throughout the year.

Colonisation of the Shell.

For the first three months after metamorphosis the shells of the young barnacles appear to be free from infection. This usually commences when the animals are from four to six months old, i.e. in September–November, and appears first near the apex of the shell, which was the region first formed. An exception to this statement is the case, described later, of certain barnacles from their extreme upper tidal limit. In all the material examined, the first alga to infect the shell was *Plectonema terebrans*, which by November had penetrated to a depth of as much as 20–26μ. From December to February, when the animals are seven to nine months old, infection by *Hyella cæspitosa* and *Gomontia polyrhiza* follows. The former was found in specimens of this age from all four localities, but Gomontia was chiefly in the material from outside Bradda.

By April the apical region of the barnacle shells is penetrated by algæ to a depth of 40-70μ, although the basal region is still only slightly infected. From April to June the main shell growth takes place, the newly formed shell forming a sharply demarcated white zone below the infected upper region. The depth of new shell usually amounts to about half of the barnacle in these second-year animals, but there is considerable variation.

The course of events in the second year is very similar to that in the first year. Infection of the newly formed shell follows the course described above, while in the upper region the algæ which infected the shell the previous year continue to penetrate deeper, reaching by November a depth of 100–110μ. The depth of the zone of shell added in the third year is less than that added in the previous year, but infection proceeds in the same way, and by the third winter the depth of penetration of the algæ in the oldest apical regions of the shell is 200–300μ. Gruvel (1905) states that in some cases barnacles are penetrated to a depth of one to two millimetres, but this would be the whole thickness of the shell in all but the very thickest specimens found at Port Erin, where the greatest depth of penetration observed in *Balanus balanoides* was 0·3 mm.

Correlation of the Presence of Barnacle-penetrating Algæ with Level and Exposure.

With two exceptions there seems to be no correlation between the distribution of any of the species found and tidal level. The first exception is that of Ostreobium and Mastigocoleus, both of which were found only within a restricted tidal range, but this may perhaps have been due to the rarity of their occurrence. The second exception is the occurrence at the extreme top of their zone, of groups of barnacles with partially or completely uninfected shells. Such individuals are not found universally, but have been recorded in very local patches from outside Bradda, Port Erin Bay and elsewhere. In the most marked cases the shell was completely free from penetrating algæ, although there were small patches of an unidentified alga in crevices in the shell surface. In other examples, surface infection took the form of visible bands round successive areas of winter-formed shell. In barnacles collected at high water at Pooylvaaish, and which, from the number of these annual zones, appeared to be three or four years old, the apical region of the shell was beginning to be infected.

The time of commencement of infection does not seem to depend on tidal level. There is, however, a marked correlation between the abundance of some of the species and the degree of wave-exposure of the locality in which they occur. Gomontia is more abundant in the very exposed habitat outside Bradda than it is in the more sheltered ones, and both Ostreobium and Mastigocoleus were found only outside Bradda. On the other hand, Hyella, which is common in all localities, is most abundant in the two sheltered Port St. Mary localities, perhaps because other algæ are less abundant in the shells there, and competition is less as a result.

Disintegration of the Shell.

Darwin (1854, p. 55) describes certain species of Balanus, as well as other barnacles, in which the diameter of the orifice of the shell is normally increased to keep pace with growth, by a process of disintegration of the tips of the lateral plates. The instances cited by him are ones in which these plates either do not separate at all at their sutures as they grow, or else do not separate sufficiently. There is no mention of such a process taking place in the present species, nor would there seem to be any need for it, since the plates are fairly readily separable. But the evidence of the size of the white zone of new growth, relative to the infected previous year's growth, seems to show a disproportionately large growth in the second year, and one not in agreement with the growth rates as already determined for the species (Moore, 1934). A simple explanation lies in the fact that the observed growth rates refer to the final size of the shell, and

take no account of any erosion that may have taken place ; and that in fact a considerable amount of such erosion does take place, especially in the thin first-year shell. This would necessitate the renewal of some of the shell in order to maintain the same size, over and above the amount required in the process of increase in size, the two together producing the white zone of total growth seen at the end of the summer. Examination of sections shows that such is probably the case since the tips of the lateral plates are frequently infected through their entire thickness, and by such a quantity of algæ that very little shell remains. This must weaken the shell so considerably that it is hard to see how it could do other than crumble away at the edge. Examination of the larger barnacles on the shore also confirms the fact that the existing apical edge is not that of the first formed post-metamorphosis plates. For this reason the width of the infected area of the first year must not be used in a subsequent year as a measure of the size at the end of the first year.

Relation between the Algæ and the Barnacle.

The infecting algæ are living in a part of the shell which apparently contains no animal tissue. There is thus no direct contact between the plant and the animal, and they cannot engage in direct transfer of salts or dissolved gases. On the other hand diffusion may perhaps take place through the shell to some extent, and also the alga will liberate metabolic products into, and withdraw others from the water immediately surrounding the barnacle. So it may be assumed that in addition to the protection afforded by the shell, the alga also benefits from the excretory products of the barnacle. The alga will in return benefit the barnacle by removing its waste products from the water, and will, in the daytime, contribute a certain amount of oxygen to the animal. The extent of the latter factor can be judged by watching a barnacle on a sunny day, when its surface is continuously giving off small bubbles of oxygen produced by photosynthesis of the algæ in the shell.

We wish to express our indebtedness to Dr. M. Knight for helpful criticism of this paper.

REFERENCES.

Bornet, M. M. 1887. Revision des Nostocacées Heterocystes. Ann. Sci. Nat., Sér. 7, T.V. Bot.

—— 1888. Note sur deux nouveaux genres d'algues perforantes. Journ. de Botanique, T. II, p. 162.

—— 1889. Sur quelques Plantes vivant dans le Test calcaire des Mollusques. Bull. Soc. Bot. France, T. XXXVI.

Cotton, A. D. 1912. Clare Island Survey, Marine Algæ. Pt. 15. Proc. Roy. Irish. Acad., Vol. XXXI.

Darwin, C. 1854. A Monograph of the Sub-class Cirripedia. The Balanidæ, the Verrucidæ. London Ray Soc.

Gruvel, A. 1905. Monographie des Cirripèdes, ou Thécostracés. Paris.

Moore, Hilary B. 1934. The Biology of *Balanus balanoides*. I. Growth and its relation to Size, Season and Tidal Level. Journ. Mar. Biol. Assoc., N.S., Vol. XIX, No. 2, p. 851.

Newton, L. 1931. A Handbook of the British Seaweeds. Brit. Mus. (Nat. Hist.), London.

Tilden, J. 1910. Minnesota Algæ. Vol. I. Rept. of the Survey. Bot. Ser. VIII, Minnesota.

The Growth Rate of *Balanus hameri* (Ascanius).

By

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With 4 Figures in the Text.

Material.

In December, 1933, a cluster of *Balanus hameri* which had been taken by fishermen on long lines set for cod in twenty-five fathoms on the outer edge of Bank Charles, about four miles south of Spanish Head, in the Isle of Man, was sent in to the Biological Station at Port Erin. The species appears to be fairly common in that locality. The cluster had started as a single barnacle, on which others had settled, and others again on these, up to, apparently, five successive broods. In all there were sixty barnacles in the cluster, and the present paper comprises an analysis of their size in an attempt to arrive at their growth rate.

In addition to the above material there was another cluster preserved in alcohol in the station museum, but unfortunately without a date. The latter was, however, known to be about 1925, and must have been between December and March, these being the limits of the cod fishery on that ground. This also was confirmed by the presence of early larvæ in the mantle cavities, while all the 1933 material was ripe but unspawned.

Methods.

Each cluster was drawn, and the individual barnacles were numbered. The opercular plates of each were then removed, and the scutum measured. A representative series of animals were removed from their shells and fixed in Bouin's fluid for sectioning. The whole cluster was then boiled for a few minutes and cleaned out, after which it was oven dried. The internal volume of each barnacle was measured by filling its shell with water from a graduated burette, and in a series of specimens the external volume also was estimated by displacement after the shell had been filled with paraffin wax.

Size Distribution.

It was hoped that the length of the scutum might afford a reliable measure of the size of this animal which changes so greatly in shape during

its life, but this was found not to be the case. In practice the volume of the barnacle was found to be a much more satisfactory measure of size, and in the size distribution curves, the cube root of the volume is taken as a linear measure of size. The size distribution data for the two sets of material are given in Table I, and those from fifty-four specimens of the 1933 material in Figure 1. The other six specimens of this series were either removed whole for sectioning or damaged. The curve for the 1933 material shows peaks at sizes of 0·1, 0·65 and 1·3 cm., approximately

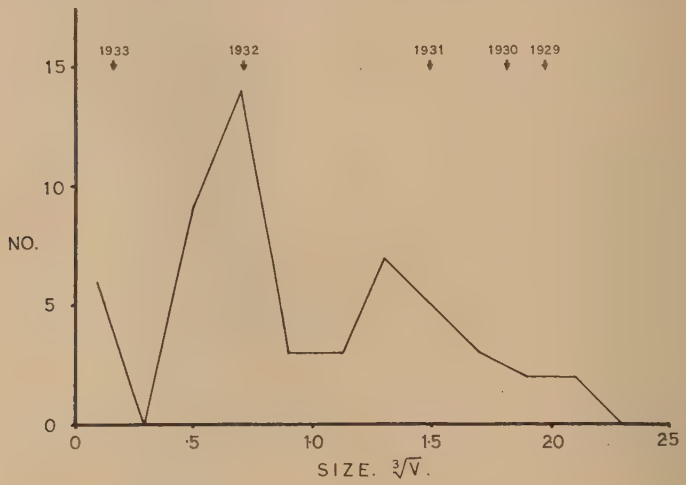

FIG. 1.—Size distribution of 1933 material. Arrows indicate mean year group sizes as obtained from successively settled broods.

($\sqrt[3]{V}$). The 1925 sample shows peaks at about 0·4, 0·9 and 1·3 cm., the increased size at which the peaks occur probably representing growth between the seasons of the two samples, but the number of available specimens is too small to be certain of this.

SUCCESSIVE BROODS.

If, as is almost certainly the case, the barnacles reproduce only once in the year, then it may be taken that where one barnacle is found settled on a larger one, the latter must be at least a year older than the former. Exceptions occur where an individual has, in the course of its growth, been pushed from its original position by another barnacle, but it is usually obvious when this has happened. In Figures 2 and 3 the 1933 cluster has been divided into two, and for the sake of simplicity, a part only of each cluster has been drawn. To begin with, there were on No. 24

a number of very small individuals (not visible in the figure) corresponding to the size of the first peak in Figure 1. Some of these were removed whole for sectioning, and hence are not included in the table of size distribution. More of this size are scattered throughout the cluster, and one of them

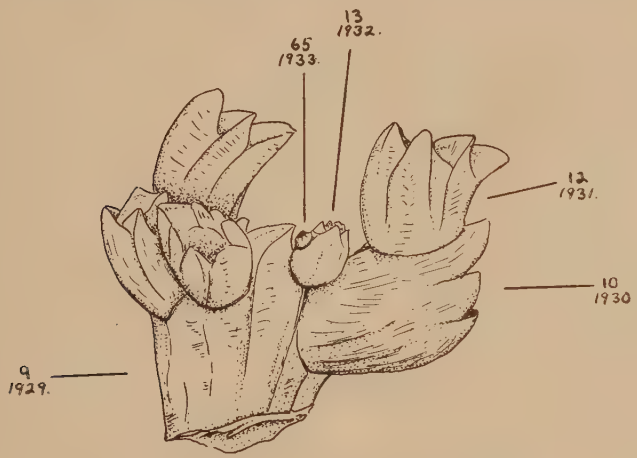

FIG. 2.—Portions of colony of *B. hameri* (1933 material) × 1.

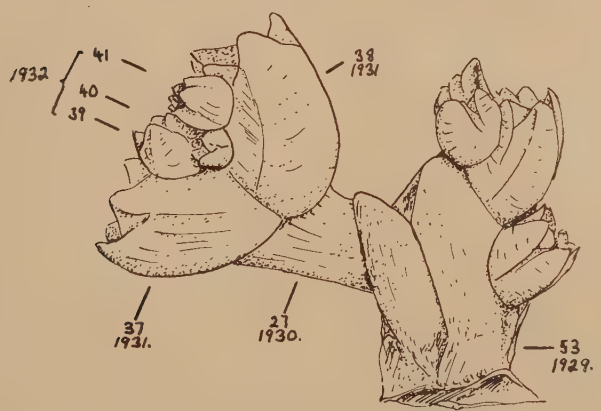

FIG. 3.—Portions of colony of *B. hameri* (1933 material) × 1.

(No. 65) is settled on No. 13 (Fig. 2) which has a size ($\sqrt[3]{\bar{V}}$) of 0·57 cm., and is thus dated, together with the rest of the barnacles comprising the second peak, as being of the 1932 group. This, of course, is on the assumption that no year group is completely unrepresented in the cluster. The

bulk of the 1932 peak is derived from a number of specimens (39, 40, 41, etc., Fig. 3), all of about the same size as No. 13, and clearly all of last year's hatching. The larger barnacles (Nos. 28, 29, 37, 38, 49, etc.) on which this 1932 group is clustered, also fall into a single size group agreeing with the third peak in Figure 1, and dating this as the 1931 group. This group is settled round No. 27 which must therefore be dated 1930, and this in turn is growing on No. 53 which must be 1929. A similar sequence is afforded by Nos. 12, 10 and 9 (Fig. 2), the latter being settled alongside No. 53 on a still larger barnacle which was dead when taken.

FIG. 4.—Growth of *B. hameri*.

The results for all those animals whose age can be determined in this way are brought together in Table II, and they yield group mean volumes as follows : 1933, 0·04 c.c. ; 1932, 0·26 c.c. ; 1931, 3·29 c.c. ; 1930, 5·90 c.c. ; 1929, 7·67 c.c. The sizes ($\sqrt[3]{V}$) corresponding to these are shown by arrows in Figure 1, and are seen to agree well with the size distribution peaks.

In Figure 4 are plotted the volumes in relation to age, taking the data from Table I for the first three years, and those from Table II for the succeeding years, as being more accurate. The biggest barnacle measured in this material had a volume of 17 c.c., and a probable age of 8 or 9 years (1925 material), but probably even larger sizes may be attained on this ground. A specimen taken growing on Keppel Pier in the Clyde had a scutum length of 29·4 mm., a height of 5·7 cm., and an external volume of

about 45 c.c. Finally, for comparison of these results with those for other species of Balanus in which it has been possible to measure external volumes only, the following ratio was obtained from a series of specimens of *B. hameri*, $\frac{\text{External volume}}{\text{Internal volume}} = 1{\cdot}5$.

Condition of the Gonads.

Of the material collected in December, 1933, twenty individuals were sectioned, comprising two first year, seven second year, nine third, one fourth and two fifth-year barnacles. In the first-year (1933) individuals no development of the gonad, either ovary or testis, was discernible. Considering the condition of the gonads in older individuals, it may therefore be said that this species does not spawn in its first season. In the second year group all individuals examined had testicular cæcæ full of ripe spermatozoa together with developing spermatocytes, and the vesiculæ seminales also were full of sperm. The ovaries were medium sized, and contained a few large, and numerous smaller ova. All the older individuals were in the same condition except for the much greater development of their ovaries, which in the old deep-shelled specimens reached a very large size. By comparison with the gonads of *Balanus balanoides* (Moore, 1934), it would seem that shedding of the genital products into the mantle cavity, and fertilisation, would probably have taken place within two or three months at most. The 1925 material showed early larvæ in the mantle cavities of all the mature specimens, thus again indicating shedding of the genital products into the mantle cavity some time between December and March. This is later than in the case of *B. balanoides* from the Isle of Man, where fertilisation usually takes place about November.

SUMMARY.

1. Sixty specimens of *B. hameri*, of five successive year groups, were obtained in December, 1933, and a further 33 preserved specimens taken in the winter of 1925 were examined.

2. The mean ages contained for successive year groups from the size distribution and from individuals settled on those of the preceding year, yielded results in good agreement with one another.

3. First-year individuals do not spawn. All older individuals had ripe genital products in the 1933 material, and early embryos in the 1925 material.

REFERENCE.

MOORE, H. B. 1934. The Biology of *Balanus balanoides*. I. Growth Rate and its Relation to Size, Season and Tidal Level. Journ. Mar. Biol. Assoc., N.S., Vol. XIX, No. 2, p. 851.

TABLE I.

SIZE DISTRIBUTIONS OF THE 1925 AND 1933 MATERIAL IN TERMS OF CUBE ROOT OF VOLUME.

Read to the nearest 0·2 cm.

Size.	Number 1925	1933
0·1	0	6
0·3	4	0
0·5	2	9
0·7	3	14
0·9	6	3
1·1	2	3
1·3	6	7
1·5	1	5
1·7	2	3
1·9	1	2
2·1	0	2
2·3	2	0
2·5	1	0
2·7	0	0

TABLE II.

Data from Successively Settled Broods.

Year group.	Specimen No.	Tergum length mm.	Mean Tergum length mm.	Internal volume c.c.	Mean Internal volume c.c.	$\sqrt[3]{\text{mean vol.}}$ c.c.	No. of specimens.
1933	65	3·2	3·2	0·04	0·04	0·16	1
1932	13	7·0	6·6	0·20	0·26	0·71	11
	20	6·6		0·15			
	39	9·0		0·37			
	40	5·1		–			
	41	7·0		0·68			
	43	3·9		0·11			
	44	6·5		0·28			
	45	8·0		0·43			
	47	6·6		0·28			
	50	6·9		0·25			
	51	6·5		0·25			
1931	12	16·3	16·5	2·85	3·29	1·49	6
	28	16·9		3·55			
	29	15·4		2·50			
	37	18·0		4·05			
	38	18·9		5·10			
	49	13·6		1·70			
1930	10	18·0	17·8	6·10	5·90	1·89	2
	27	17·5		5·70			
1929	9	21·7	21·2	7·25	7·67	1·97	2
	53	20·7		8·10			

The Larval Stages of *Balcis alba* and *B. devians.*

By

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Naturalist at the Plymouth Laboratory.

With One Plate.

The young stages of the members of the family Eulimidæ are little known, the only reference to any species apparently being that of Loven (1844) who briefly notes and figures a veliger which he attributes to *Eulima distorta* (=*Balcis devians*). This has a very oblique shell and was probably a week or two old. A comparison of this figure with the apex of *Balcis devians* from Plymouth and its larvæ in the plankton makes one rather uncertain of its identity but shows that even in those early times it was known that a Eulima could remain some time in the plankton as a veliger.

EULIMIDÆ.

Genus **Balcis.**

Balcis alba (da Costa) (=*Eulima alba*).*

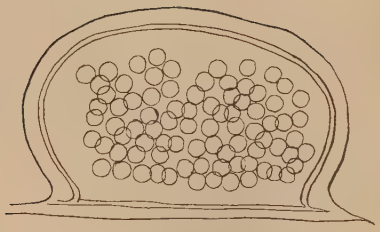
(Plate I, Figs. 1–10.)

This is the commonest species of eulimid at Plymouth, being dredged frequently on the Mewstone Amphioxus ground, Mewstone Ledge and beyond the Eddystone. Several specimens were collected and placed in a plunger jar on the glass sides of which some of them laid eggs. The eggs are described here for the first time. The first batches were laid on March 10th, 1932, after which several more were deposited from April throughout the summer and also in the following years. No spawn has been seen in its natural surroundings.

The spawn case, or oötheca (Plate I, Fig. 1) is enormous for the size of the animal, measuring about $3 \times 2{\cdot}5$ mm., the adult shell rarely reaching a length of $\frac{3}{4}$ inch. Oval, opaque with a very thick and tough skin of two layers, white, rounded on the free surface, flat on the under surface which is attached to some substratum, in this case the glass of the plunger jar. Inside are some hundreds of eggs although when newly laid they do not

* The names follow Winckworth's (1932) list, and the Plymouth Fauna List names (Marine Biological Association, 1931) are in brackets. Winckworth (1934) confirms his names in his later paper.

PLATE I.



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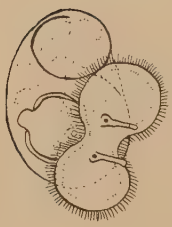

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nearly fill it, the space being filled with a liquid which is absorbed by the developing eggs. Eggs pinkish, at first about 0·10 mm. across, rapidly enlarging to about 0·16 mm. and hatching in about three weeks. Eggs laid on March 10th hatched on April 5th, other batches hatched in rather less time, some took rather longer. The newly hatched young were removed from the original plunger jar and placed in a fresh one with some pure Nitzschia culture for food. Unfortunately none of them lived for more than a few days. It was, however, possible to see the characters of the larvæ and compare them with those from the plankton. In this way one could distinguish them from those of closely related forms. The larvæ are common in the plankton, usually from outside the Sound, but occasionally from inside. The late veligers in particular occur frequently in summer (especially August and September) between stations L4 and L5 (in direct line towards the Eddystone) and over the Eddystone and Mewstone grounds. They remain for a long time as veligers attaining five whorls at least before losing the velum and retiring to the bottom.

The newly hatched larva (Plate I, Figs. 2, 3) measures about 0·16 to 0·18 mm. across the shell and has about $1\frac{1}{2}$ whorls. The apex is very broad and the larval shell resembles closely that of *Pelseneeria stylifer* (=*Stilifer stylifer*) hatched out in the Laboratory from eggs laid on Psammechinus (see Lebour, 1932), but the apex soon becomes more pointed (Plate I. Fig. 4). Pelseneeria is evidently closely related to the Eulimidæ, A striking feature of the larval *Balcis alba* is the deep black pigment running round the mouth and up the œsophagus (Plate I, Fig. 2) which continues for the whole of the larval life, the region of the digestive gland also being dark, the alimentary canal is forming, otocysts, eyes, tentacles, foot and operculum are conspicuous and the velum, measuring about 0·2 mm. across is bilobed with round lobes (Plate I, Figs. 2, 4), and quite colourless. The outer lip of the shell is drawn out slightly into a process supporting the velum. The veligers in the plankton are of similar form and colour, with the spire gradually lengthening (Plate I, Figs. 6–10). The foot, tinted with black or dark grey, is three lobed, the region of the digestive gland dark and the black lines running towards the mouth very

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EXPLANATION OF PLATE.

PLATE I.

Fig. 1.—Spawn of *Balcis alba*, laid on glass of aquarium, 3 mm. across.

Fig. 2–3.—Newly hatched larva of *Balcis alba*, 0·16 mm. across shell (dorsal view).

Fig. 4–5.—Slightly older larvæ (ventral view).

Fig. 6–10.—Late larvæ of *Balcis alba* from plankton shell 0·66–0·72 mm. long.

Fig. 11–12.—Young larva of *Balcis devians* from plankton shell 0·24 mm. long.

Figs. 13–14.—Late larvæ of *Balcis devians* from plankton shell 0·64 mm. long.

Fig. 15.—Apex of adult *Balcis devians*.

conspicuous. As the apex lengthens the animal withdraws from the last whorl or two of the shell so that the first two whorls are usually empty in the older larvæ. When the shell has five whorls it measures about 0·66 to 0·72 mm. in length, the dark grey foot has a long contractile fore part covering the mouth ventrally, the top of the animal below the two first whorls is dark and the velum still colourless with the lobes very round, usually unequal in size, the largest lobe being nearly equal to the length of the shell (Plate I, Figs. 6–7). At this late stage when the animal is about to metamorphose it is often found with the veligers of *Cerithiopsis tubercularis*, *C. barleei* and *Triphora perversa* (see Lebour, 1933), all of which correspond in size and form, having several whorls and bilobed velum with round lobes. The larva now loses the velum and crawls. The black pigment no longer shows in the adult which has a white body flecked with orange and yellow, especially round the head and tentacles, the foot also being white. The tip of the spire in the adult is nearly always broken off, the broken tip being filled up with calcareous matter.

Balcis devians (Monterosato) = *Eulima philippi*.

(Plate I, Figs. 11–15.)

This species is common among sponges and other sessile animals dredged in the Sound. It is well known for having a very much curved spire, but the first four or five whorls are quite straight, the curve only beginning apparently after the animal has lost the velum and taken to a life on the bottom. Larvæ attributable to this species are common in the inshore tow-nettings in spring and summer, especially July to September. They are much the same size as those of *B. alba* but without the black pigment in the animal, the spire of the shell being blunter and the apex usually not broken off in the adult as in that species. The adult is much smaller, about 10 mm. or less in length. The digestive region in the larva is a pale yellowish brown, and the same colour shows through the shell of the adult which is very thin. The late larva has four or five whorls with a colourless velum with round lobes. The larva then loses its velum and goes down to the bottom.

This is the commonest eulimid in the inshore waters. A few other larvæ have been seen but not identified. They probably belong to *Eulima pernula* (= *Strombiformis bilineata*) and *E. glabra* (= *Strombiformis glabra*) (see Winckworth, 1934). These both occur at Plymouth, but are rare. Their spires are very slender and the larvæ would differ from those described above in this respect.

Judging from the two species described both larvæ must play an important part in the plankton, being common and remaining near the surface for a long time.

LITERATURE.

LEBOUR, M. V. 1932. The Eggs and Larvæ of Two Commensal Gastropods, *Stilifer-stylifer* and *Odostomia eulimoides*. Journ. Mar. Biol. Assoc., N.S., Vol. XVIII, No. 1, pp. 117–122.

—— 1933. The Life Histories of *Cerithiopsis tubercularis* (Montagu), *C. Barleei* Jeffreys and *Triphora perversa* (L.). *Ibid.*, Vol. XVIII, No. 2, pp. 491–498.

LOVEN, H. 1844. Om nordiska Hafs-Mollusken. Oversigt af Kongl. Vetenskaps-Akademien förhandlingar, forste ărgăngen, pp. 48, 53.

MARINE BIOLOGICAL ASSOCIATION, 1931. Plymouth Marine Fauna, 2nd edition, pp. 1–371.

WINCKWORTH, R. 1932. The British Marine Mollusca. Jour. of Conch., Vol. 19, No. 7, pp. 221–252.

—— 1934. Names of British Mollusca. II. *Ibid.*, Vol. 20, No. 1, pp. 11–15.

On the Occurrence of Post-Larval Stages of the Bass, *Morone labrax* (L.), in the Plymouth Area.

By

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Naturalist at the Plymouth Laboratory.

The post-larval stages of the bass, *Morone labrax* (L.), have never been recorded from these waters. From time to time occasional specimens of young fish have been caught in the plankton which had been tentatively assigned to this species on account of their similarity to the drawing given by Raffaele (1888). Now that the post-larval stages of this species have been described and figured by Bertolini (1933, p. 310 and Plate XIX) the Plymouth specimens can be definitely identified as *Morone labrax*, and the instances of their occurrence put on record.

Among all the specimens of young fish in the collections made during the years 1924 to 1934 only fourteen specimens of young bass have been observed, their occurrence being as follows :—

Date.	Position.	Depth of haul.	Specimens.
2.iv.25	L4	29·0 m.	1 (5 mm. long.)
29.iv.25	L6	24·3 m.	1 (4 mm. ,,)
19.v.25	L4	12·9 m.	1 (6·5 mm. ,,)
19.v.25	L4	18·9 m.	1 (4·5 mm. ,,)
13.iv.26	2 miles E. of Eddystone	21·6 m.	1 (6 mm. ,,)
22.iv.26	,, ,, ,,	24·6 m.	1 (7 mm. ,,)
22.iv.26	,, ,, ,,	39·0 m.	1 (7·5 mm. ,,)
3.vi.26	,, ,, ,,	18·0 m.	1 (6 mm. ,,)
4.vi.26	,, ,, ,,	36·3 m.	1 (7 mm. ,,)
17.iii.31	,, ,, ,,	Oblique haul	1
26.iii.31	,, ,, ,,	,, ,,	1
9.iv.31	,, ,, ,,	,, ,,	1
21.v.31	,, ,, ,,	,, ,,	1 (6 mm. long.)
27.iv.33	,, ,, ,,	,, ,,	1

From this it is evident that the young stages are likely to be met with in the plankton during the months March to June, but that they are

extremely rare. The high proportion of specimens in 1925 and 1926 is because in those years many more hauls were taken during the study of vertical distribution than in the later years, when oblique hauls were taken generally about once a week ; but the preponderance of specimens in 1931 among the later years is noteworthy.

Holt and Byrne (1898) obtained unfertilised eggs from a ripe bass in one of the laboratory aquarium tanks at the end of May ; they comment on the absence of records of eggs and young in nature and suggest the possibility that the fish spawn in the estuaries. From the positions of capture of the above specimens it seems likely that spawning takes place in the open sea here as in the Mediterranean. The rarity of the young stages in our collections is also possibly no more than a reflection of the rarity of the adult bass itself by comparison with the large numbers of the commoner species of fish.

The depths of capture of these occasional Plymouth specimens indicate that in these waters the post-larval bass live in the daytime in the deeper water layers mostly below 20 metres.

REFERENCES.

Bertolini, F. 1933. Uova, larve e stadi giovanili di Teleostei. Fauna e Flora del Golfo di Napoli. Monografia 38, p. 310.

Holt, Ernest W. L., and Byrne, L. W. 1898. Notes on the Reproduction of Teleostean Fishes in the South-Western District. Journ. Mar. Biol. Assoc., N.S., Vol. V, No. 3, pp. 333–340.

Raffaele, Fed. 1888. Le uova galleggianti e le larve dei Teleostei nel golfo di Napoli. Mitth. Zool. Stat. Neap., Vol. VIII, pp. 1–84.

Digestion in the Plaice (*Pleuronectes platessa*).

By

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With 6 Figures in the Text.

The study of the digestive processes in the teleost fishes presents some interest since, although the structure of the alimentary canal is in general very similar to that of the mammalia, there may be certain important differences. For example, in many fishes there is no anatomical stomach (*Fundulus*, Babkin and Bowie, 1928), while in others there is a stomach, but no acid is secreted (*Zoarces*, Mackay, 1929a). Again, in most teleost fishes, the pancreatic tissue is scattered diffusely throughout the abdominal cavity, and it is problematical whether there is any external secretion of digestive juices, and, if there is, how the juices reach the alimentary canal. Finally, many fishes possess peculiar diverticula of the duodenum at the pylorus (the pyloric cæca), the function of which is not exactly known.

Methods.

It is impracticable, from an animal the size of the average plaice, to obtain sufficient quantities of pure digestive juices to enable one to make an adequate study of their enzyme contents. It has been necessary, therefore, to make extracts of the mucous membranes of the various parts of the digestive tract—usually with glycerol, but in some cases with 30% alcohol, and, in the case of the stomach, with N/10 hydrochloric acid—and to study the enzyme content of the extracts.

In most cases, the fish was stunned by a blow on the head, and killed by pithing, although in a few cases, the fish was anæsthetised with urethane. The whole alimentary canal was then removed, by carefully dissecting away the attachments to the mesentery, and separated into the various components; these were slit longitudinally, washed under the tap and the mucous membrane scraped off on a glass plate. The scrapings were then ground with sand, and extracted for about two days with rather more than an equal volume of glycerol or other extraction fluid (compare Mackay, 1929a). The extracts were then diluted with N/20 HCl (stomach) or N/20 NaOH (intestine), strained through muslin and made up to the volume required for the tests. This was usually about 1 ml. per 100 g.

fish, but the actual dilution varied on different occasions. The addition of acid or alkali facilitated the filtration, and brought the extract to approximately the appropriate acidity for the tests; it also prevented the proteins from coagulating when the extracts were boiled to destroy the enzymes. The protein concentration of the extracts varied, but lay between 0·1% and 0·5%.

The solutions were buffered during digestion, each tube containing 1 ml. of the extract, 1 ml. of M/5 buffer, and 1 ml. of the substrate solution. Since the extracts were themselves somewhat buffered, the final value of the acidity was different from that of the original buffer solution; it was measured when necessary, with the quinhydrone electrode.

Digestion was carried out in a water bath at 25·5°±0·15°. Hitherto, most studies of enzymes from cold-blooded animals have been carried out either at 37° or at room temperature. It was felt that a temperature as high as 37° might introduce abnormal effects, while the reactions would proceed so slowly at room temperature that significant effects might be missed: a compromise was therefore preferred. There would appear, however, to be no evidence that the enzymes behave in any way differently at 37°, room temperature, or 25°.

When comparing the enzymic activities of different organs, it was felt that it was more satisfactory to compare, for example, the lipoclastic activity of all the liver from a kilogram of fish, with that of all the intestinal mucous membrane, rather than to compare the activity of a gram of liver with a gram of intestinal mucous membrane.

The Digestion of Proteins.

Methods. Northrop (1932) recommends the following methods for the study of peptic activity: (1) the change in viscosity, (2) the decrease in protein nitrogen, and (3) the decrease in peptide linkages (formol titration); gelatin, casein and edestin may be used as substrates. At the beginning of this work the method involving carmine fibrin as substrate was used; later this was replaced by a modification of Northrop's method (2) and by the formol titration method (3); method (1) was not used.

While the carmine fibrin method is satisfactory so long as the range of hydrogen-ion concentration is restricted to pH less than 4 or so, it is useless in alkaline solutions (for testing for trypsin for example), since the carmine is dissolved off the fibrin even in the absence of any proteoclastic action. Vonk (1927) recommends the use of fibrin stained with Spirit Blue (diphenyl-rosaniline) for investigating tryptic activity, and, after studying the properties of a number of similar dyes available in the laboratory, gentian violet was found satisfactory. This was only used for a few experi-

ments, however, since it was found that bile dissolved it off the fibrin more rapidly after boiling than before, and, on one occasion, intestinal extract behaved similarly. It was found also, that the amount dissolved off by the boiled extract depended upon the acidity both with gentian violet and carmine, so that a separate control was necessary for every value of acidity studied.

When this method had been rejected, Northrop's method (2) was adopted, using 2% casein or normal horse serum diluted to one-fifth, as substrates. The coagulable protein was estimated initially, and after a suitable period, in the digests and in controls containing boiled extract, by the method of Kerridge (1931). This consists in adding a known proportion of a suspension of India ink and then precipitating both protein and ink with trichloracetic acid. The greyness of the resulting suspension is then compared, by reflected light, with that of standard protein solutions similarly precipitated.

Polypeptidases, and in some cases proteinases, were estimated by formol titration, peptone being used as substrate for the former. One drop of 0·1% phenol phthalein solution was added to each ml. of the digest, and the whole, or an aliquot part, titrated to a definite pink colour with N/50 NaOH containing one drop of the phenol phthalein solution per ml. 0·1 ml. of 40% formol (neutralised to phenol phthalein) was then added to each ml. and the solution titrated to the same colour as before. To facilitate this, the digests were titrated in pairs (usually " active " and " boiled control ") both being brought to the same pink colour ; formol was then added to the first, which was titrated to the colour of the second, whereupon formol was added to the second, and this titrated to the colour of the first.

This proved to be the only satisfactory method of investigating the proteoclastic activity of the bile, since its colour precluded the use of the Kerridge method ; moreover, its surface activity resulted in a different degree of granularity of the precipitate on different occasions, which made the comparison even more difficult.

RESULTS.

A. The Stomach.

That extracts of the mucous membrane of the stomach digest protein in acid solution has been shown by all three methods. The optimum pH appears to lie between 1·5 and 2·5, and no digestion takes place in solutions alkaline to pH 5·5. Proteins do not appear to be broken down beyond the polypeptide stage, since the extracts have no action on peptone (formol titration method, Table I). No difference could be detected between the pH optima of the glycerol extracts and of the acid extracts, but it must

be admitted that, possibly owing to the technique employed, there appeared to be no clear-cut and regular influence of the hydrogen-ion concentration in either case between pH 1·5 and pH 3 (Fig. 1). The absence of any activity at acidities less than pH 5·5, and the similarity between the behaviour of the acid and glycerol extracts, indicate the absence of considerable quantities of cathepsin. These extracts appear to differ in this respect from those of mammalian gastric mucous membrane studied by Willstätter and Bamann (1928), which regularly contained both cathepsin and erepsin, greater quantities being present in glycerol extracts than in acid extracts.

The stomachs of fishes 78, 81, 90 and 93, to which pilocarpine had been administered for some hours (see later), were found to have secreted

TABLE I.

Proteoclastic Action of Extracts of Mucosa of Stomach and Intestine, and of Bile obtained by Slitting Gall-bladder.

Difference between the formol titration figures (ml. N/50 NaOH) of solutions containing 1 ml. buffer (pH 2 for stomach and pH 8 for intestine and bile), 1 ml. substrate and 1 ml. extract, and similar solutions containing boiled extract, after 23 hours at 25°.

Substrate.	Stomach.		Intestine.				Bile.	
			Proximal 25 mm.		Distal 285 mm.			
	Total Volume of Extract ml.	ml. N/50 NaOH.	Total Volume of Extract ml.	ml. N/50 NaOH.	Total Volume of Extract ml.	ml. N/50 NaOH.	Dilution.	ml. N/50 NaOH
Horse serum	4·8	0·15	6·0	− 0·02	5·0	0·01	4	0·13
Peptone		− 0·02		0·40		1·02		0·14

appreciable quantities of a clear viscid fluid. From the stomachs of Nos. 81, 90 and 93 were obtained sufficient quantities of this fluid to enable it to be tested for proteoclastic activity. In all cases it was found that casein was digested at pH 2, but not at any pH greater than 5·5. A slight action was observed with the extract from No. 93 at pH 5·25 and a definite action with that from No. 90 at pH 5·0. All these specimens of gastric juice were acid (pH 5·60, 3·85 and 6·45), and those from fishes Nos. 90 and 93 contained 0·445 M. and 0·27 M. chlorides (equivalent to 2·6% and 1·6% NaCl respectively). It is probable, therefore, that appreciable quantities of sea-water had leaked in through the cardiac sphincter. There is no doubt, therefore, that pepsin can be secreted into the lumen of the stomach.

B. The Intestine.

The plaice has no anatomically distinct pancreas, but according to Cole and Johnstone (1901), pancreatic tissue is distributed along the walls

of the blood vessels of the mesentery, around the paired pyloric cæca (from which small ducts enter the intestine) and embedded in the liver, surrounding the smaller branches of the portal vein. It is thus impossible to remove the mucous membrane from the inside of the cæca (and the

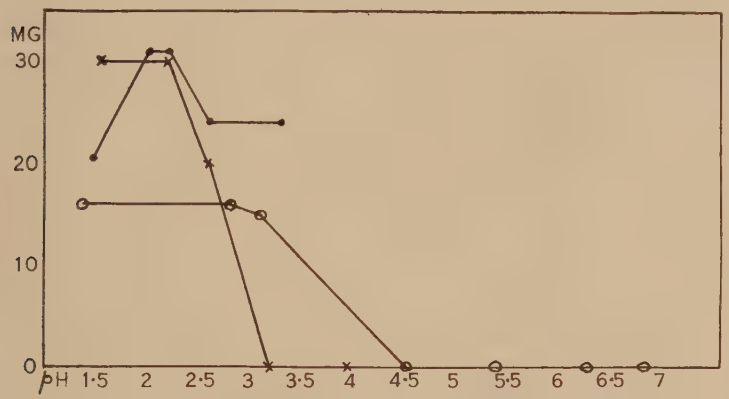

Fig. 1.—pH-Activity Curve of Proteinase from Mucous Membrane of Stomach.

Ordinates: mg. protein digested per hour by extract of whole mucous membrane of stomach from 1 kg. of fish.

● Expt. 73/74. Acid extract. *Substrate:* casein. Duration of digestion: 1·2 hours.

× Expt. 32/34. Glycerol extract. *Substrate:* horse serum. Duration of digestion: 3·3 hours.

⊙ Expt. 35/36. Glycerol extract. *Substrate:* horse serum. Duration of digestion: 11 hours.

neighbouring parts of the duodenum) without contamination with pancreatic tissue from the outside, the cæca being too small to be cleanly dissected away.

In many fish, the most distal of the four cæca could not be identified. Measurements made on those in which it was prominent, showed, however, that it could be presumed to lie within the proximal 10% to 15% of the intestine.

By analogy with the mammalia, then, one might expect that the mucous membrane obtained from the region of the intestine near the cæca would contain a trypsin-like enzyme, while the more distal portions would contain only an erepsin-like enzyme.

In experiments made in June and July, this was indeed found to be the case. Thus in Expt. 28, the extracts from the mucosa of the upper 3 cm. of the intestine, and those from that of the remaining 18 cm., were each made up to 6 ml. 1 ml. of the former digested 21 mg. of casein in 24 hours, while 1 ml. of the latter had no detectable action whatever at any acidity between pH 6·8 and pH 8·7. Later, however, in August and

September, these results could not be confirmed, there being no detectable quantities of trypsin in either portion. In October, trypsin was found in small quantities in the distal regions, but none in the proximal. No explanation for this discrepancy can be advanced. In the early experiments, the analyses were made by the Kerridge method, using casein as substrate ; in the later ones, both this method and the formol titration method were used, and normal horse serum was used as substrate in addition.

The optimum pH of this enzyme lies between 7·5 and 8·5, as will be seen from Figures 3 and 4. There would appear to be cathepsin present

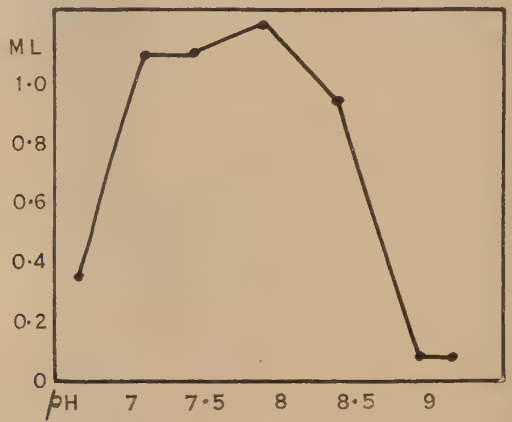

Fig. 2.—pH Activity Curve of Polypeptidase from Mucous Membrane of Intestine.

Ordinates : Decrease in peptide linkages (formol titration) produced per hour by whole extract of mucous membrane of 1 kg. of fish.
Substrate : casein. Duration of digestion : 16 hours.

in some of the extracts, in addition to the trypsin, but the amount, in relation to that of the trypsin, is very variable.

An erepsin-like enzyme is present in relatively large quantities in the mucous membrane of all parts of the intestine, whose optimim pH lies between 7·5 and 8·0 (Fig. 2).

C. Bile.

Babkin and Bowie (1928) found that the bile of *Fundulus heteroclitus*, as obtained from the gall-bladder, contained a protease, a lipase and an amylase. Mackay (1929b), however, found that the bile of this fish was free from enzymes if, before puncturing the gall-bladder, the pancreatic tissue surrounding it was destroyed by immersion in Bouin's fluid for one minute.

In the plaice, also, the bile has been found to contain a protease closely resembling the trypsin of the mammalian pancreas. It is activated by extracts of the intestinal mucous membrane, having, by itself, little or no action, and has an optimum pH between 7·5 and 8·5 (Fig. 3). Polypeptidases, also, were definitely present in most samples of bile obtained by slitting the gall-bladder (compare Table III).

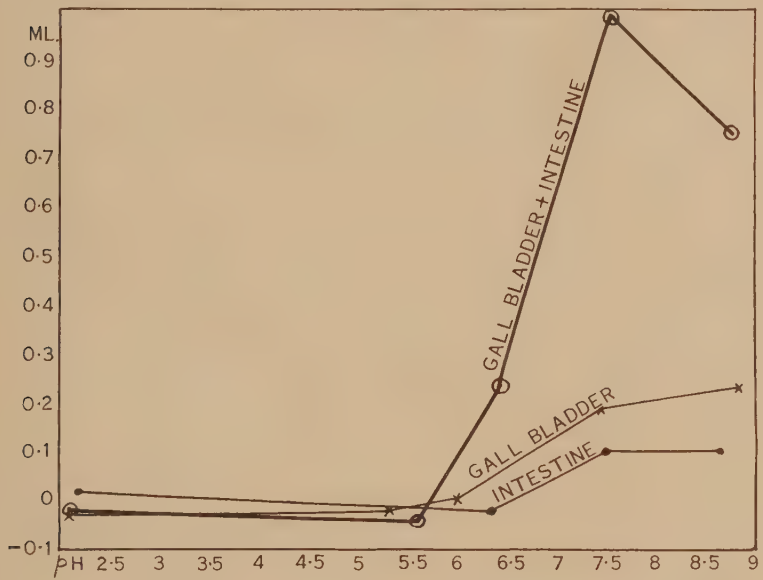

Fig. 3.—pH-Activity Curve of Proteinases in Glycerol extract of Intestinal Mucous Membrane and in Bile extract of Gall-Bladder.

Gall-bladder from 1145 g. fish. Volume of Bile: 11 ml. Intestinal extract from 570 g. fish. Volume of extract: 12 ml.

Substrate: casein. *Ordinates:* decrease in peptide linkages (formol titration) in 8·2 hours in terms of ml. N/50 NaOH.

● Action of intestinal extract alone.
× Action of gall-bladder extract alone.
⊙ Action of mixture of both in equal proportions.

Mackay's (1929b) experiment was repeated and confirmed, the gall-bladder in this case being dropped into Zenker's fluid for about 5 seconds; bile obtained by slitting the wall of the gall-bladder after fixation of the pancreatic tissue surrounding it, was free from proteoclastic activity.

In the plaice, however, a more crucial method is available, since the bile duct can be cannulated and the bile obtained without damage to the wall of the gall-bladder. Such bile was uniformly devoid of proteoclastic action. If, on the other hand, the bile is allowed to extract the gall-bladder for 1 to 2 days, an extremely active preparation is obtained

(Table I). That the enzyme concerned is truly a trypsin, and not a cathepsin—which might be found in any tissue—is shown by Expt. 94/98 (Fig. 3). It can be seen (1) that the mixture of gall-bladder extract with intestinal extract is very much more active than either alone, and (2) that the greatest activity in all the extracts occurs between pH 7·5 and pH 8·5 (the complete inactivity of the intestinal extract in solutions more acid

TABLE II.

Proteoclastic Action of Bile.

Difference between the Formol Titration figures (ml. N/50 NaOH) of solutions containing 1 ml. diluted bile, 1 ml. buffer pH 7·5–8, 1 ml. of substrate solution, and 1 ml. of intestinal extract (fresh or boiled), and of similar solutions containing boiled bile, after 24 hours at 25°.

Fish Number.	Total Weight, grams.	Total Volume of Intestinal Extract.	Dilution of Bile.	Substrate.	Activity (ml. N/50 NaOH) due to Bile alone.	Activity (ml. N/50 NaOH) due to Bile in presence of Intestinal Extract.	Activity (ml. N/50 NaOH) due to Intestinal Extract alone.
		c.c.		A. Uncontaminated Bile.			
(51, 53)*	535	6	5	Horse serum	0	0·05	0·10
57‡	390	–	–	,, ,,	0	–	–
61, 62, 63†	940	13	5	Casein	0	0·05	0·32
(66, 67)†	665	8	4·7	,,	0	0	0·35
68†	655	10	4·5	,,	– 0·05	– 0·05	0·20
(69, 70)‡	380	5	6	,,	–	–	0·15
72†	765	5·5	4·5	,,	– 0·10	0	1·20
				B. Bile obtained by Slitting Gall-Bladder.			
42	750	11	4	Horse serum	0·13	–	0
				Peptone	0·14		1·4
44, 46	430	14	5	Horse serum	0·10	–	0
				Peptone	0	–	0·90
48, 49	510	8	5	Horse serum	0·06	0·68	0·10
59	930	15	7·5	Casein	0	0·12	0·25
				C. Bile allowed to Extract Gall-Bladder.			
40, 41	770	6·5	4	Horse serum	0·10	–	–
				Peptone	1·45	–	3·05
50, 52	550	6	5	Horse serum	0	0·90	0·10
54	265	–	5	Horse serum	–	0·65	–
68	655	10	4·5	Casein	0·05	1·85	2·0

to pH 6·5 is unusual, as there is more often a small quantity of cathepsin present—see Fig. 4). It is much more likely that the gall-bladder extract is activated by the enterokinase in the intestinal extract, than that the intestinal extract is activated by some substance in the gall-bladder.

It would appear, then, that pure bile has no proteoclastic activity, but that the layer of pancreatic tissue surrounding the gall-bladder is capable

* Gall-bladder fixed in Zenker's fluid.
† Bile obtained by cannula in bile-duct.
‡ Bile obtained by cannula, after acid had been inserted in intestine.

of secreting a trypsin-like enzyme. There does not appear to have been any special duct described, through which this pancreatic juice could reach the intestine, and it is tempting to suppose that secretion takes place into the gall-bladder and that the enzyme reaches the intestine in the bile. If this is the case, it should be possible to make pure bile (as obtained by cannulating the bile duct) active in digesting proteins, by treating the fish with pilocarpine. This experiment has been performed eight times, the fish being on an artificial circulation of sea-water, and under urethane as described later. The bile duct was ligatured at the beginning of the experiment, between the entry of the hepatic ducts and the intestine in four

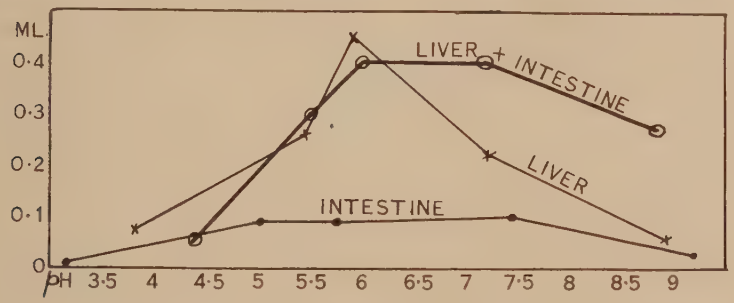

FIG. 4.—pH-Activity Curve of Proteinases from Glycerol extracts of Intestinal Mucous Membrane and of Liver.

Total weight of fish: 745 g. Volume of intestinal extract: 12 ml. Volume of liver extract: 17 ml. *Substrate:* casein. *Ordinates:* decrease in peptide linkages (formol titration) in 6·2 hours in terms of N/50 NaOH.

● Action of intestinal extract alone.
× Action of liver extract alone.
⊙ Action of mixture of both in equal proportions.

experiments, and at the base of the gall-bladder in four experiments; in the former, bile secreted by the liver during the experiment, possibly containing pancreatic juice, would be included in the sample of bile collected; in the latter, it would not. At the end of the experiment, the bile duct was cannulated close to the base of the gall-bladder, and the bile expelled by gentle pressure with the fingers. The pilocarpine (as nitrate or chloride) was added to the circulating sea-water (total volume 300 to 400 ml.), and its effectiveness was checked by counting the frequency of the heart beat.

It is difficult to expose the heart of the plaice sufficiently for direct visual observation, so an electrode was placed on the pericardium and another either under the tail or in the gill cavity, and the action potential waves were counted. Since it was not necessary to make distortionless records, the leading off electrodes were connected to a three-stage amplifier with a milliammeter in the anode circuit of the last stage; the time for twenty kicks of the milliammeter needle was measured with a stop-watch. It was sometimes necessary

to stop the flow of water through the gills while the heart frequency was being measured, owing to the large slow potential waves associated with the rhythmic flow of water through the gills. The exact origin of these waves has not yet been ascertained. One fish showed a marked and progressive slowing of the heart when the circulation through the gills was stopped, but this was not observed in any other fish.

One experiment was performed without the addition of pilocarpine. The heart frequency immediately after the operation was 51 beats per minute ; it rose to 57 within five minutes, fluctuated, during the course of the experiment, between 52 and 60, and was 54·5 after five hours on the artificial circulation.

The results of the analyses of the bile obtained in these experiments are

TABLE III.

Action of Pilocarpine on Proteoclastic Activity of Bile.

Conditions of digestion experiments as in Table II. Substrate : Casein.

Fish Number.	Weight grams.	Total Volume of Intestinal Extract.	Dilution of Bile.	Dose of Pilocarpine mg.	Frequency of Heart beats per min. Initial.	Frequency of Heart beats per min. Final.	Activity (ml. N/50 NaOH) due to Bile alone.	Activity (ml. N/50 NaOH) due to Bile in presence of Intestinal Extract.	Activity (ml. N/50 NaOH) due to Intestinal Extract alone.
71	513	7·5	6·5	6	50	38·5	0·13	0·15	0·20
75	495	–	–	10	47·5	35	– 0·02	0	– 0·12
78*	340	5	6·5	13	47	24	– 0·07	– 0·05	0·13
81	460	5	4·5	13	48·5	0	0·05	0·10	0·10
88*	580	7	2·2	13	Not measured	32·5†	0	0·10	0·60
89*	545	6	3·8	13	51·5	41	– 0·10	0	0·45
90	490	7	3·0	13	46·5	31	– 0·06	0·06	0·25
93*	900	10	4·5	26	54·5	38·5	– 0·03	0·10	0·4

given in Table III. While it is possible that in four of these pilocarpine experiments (two including the hepatic secretion, and two not) the bile had a detectable proteoclastic action, the effect is barely outside the limits of experimental error. It must be concluded, therefore, that no definite evidence has been obtained that a proteoclastic enzyme is secreted into the bile in response to the administration of pilocarpine.

D. Liver.

Glycerol extracts of the liver contain both cathepsin and trypsin, as is shown in Figure 4, which presents a striking contrast to Figure 3. In the absence of intestinal extract, the cathepsin is in preponderance (optimum pH 5·5 to 6·0) ; in the presence of intestinal extract, the trypsin (optimum pH about 8) is activated, and the optimum pH of the mixture

* Duct ligatured below entry of hepatic ducts, i.e. hepatic bile not excluded.
† By direct examination after opening pericardium.

is shifted to about 7. Stern (1931), who studied the proteases of the liver of the carp, demonstrated the presence of a cathepsin with an optimum pH of 4 and of a trypsin with an optimum pH of 10, so that the pH-activity curve contained two distinct peaks. He did not activate the trypsin with enterokinase, and studied the proteinases only, estimating the amount of digestion by a nephelometric method after precipitation with sulphosalicylic acid. The formol titration method, used in the present series, would estimate polypeptidases also, and the difference in the shape of the pH-activity curve may be connected with this difference in technique.

The Digestion of Fats.

Methods. The method used was essentially that described by Anrep, Lush and Palmer (1925). The only modification was that at suitable intervals, the experimental tube, containing the active enzyme, was titrated with N/5 NaOH to the same pH as the control tube, containing the boiled enzyme. In this way the turbidity and colour of the extract were compensated, and the hydrogen-ion concentration was not allowed to drift out of the optimum range of the enzyme. The figures obtained in this way for the activities of the various extracts were also independent of the buffer value of the digests.

RESULTS.

A. Stomach.

Dawes (1930) states that histological examination of the walls of the stomach of the plaice after feeding shows that absorption of fats takes place from the stomach. Accordingly, the extracts of the mucous membrane of the stomach were examined for lipoclastic activity. None could be found at pH 2·5, 4·5 to 6·0, or 7·5 to 8·5, at any season of the year.

B. The Intestine.

Extracts of the mucous membrane of the intestine showed a powerful lipoclastic activity, which was increased by the addition of bile. The optimum hydrogen-ion concentration lay between pH 7·5 and pH 8·0, although the activity does not vary very considerably over the range pH 6 to pH 9 (Fig. 5). The portion distal to the cæca showed a greater activity than the portion containing the cæca, so that there is no reason to suppose that the enzyme is particularly associated with these organs (Table IV).

C. Bile.

Bile removed from the gall-bladder without any special precautions against contamination with the pancreatic tissue showed a negligible lipoclastic activity. The plaice appears to differ in this respect from

Fundulus (Babkin and Bowie, 1928). Bile which has been allowed to extract the gall-bladder, however, showed on one occasion an activity comparable in magnitude to that of the intestine; on another occasion, there was no activity.

TABLE IV.

Lipoclastic Action of Intestinal Extracts.

ml. N/5 acid produced by 1 ml. extract in 7 hours at pH 7·5 to 8.

Three fish of total weight 715 g. Proximal portion of intestine taken as 23%, 10·5% and 10·7% of whole; last two had distal cæca. Both extracts made up to about 6 ml.

Portion of Intestine.	Without Bile.	With Bile.	Bile only.
Proximal	0·10	0·13	0·02
Distal	0·28	0·33	0·01

D. Liver.

Glycerol extracts of the liver possessed an extremely powerful lipoclastic action (Fig. 5).

The Digestion of Carbohydrates.

Methods. Preliminary studies were made with the achromic point method, in which a drop of a solution of iodine in potassium iodide is

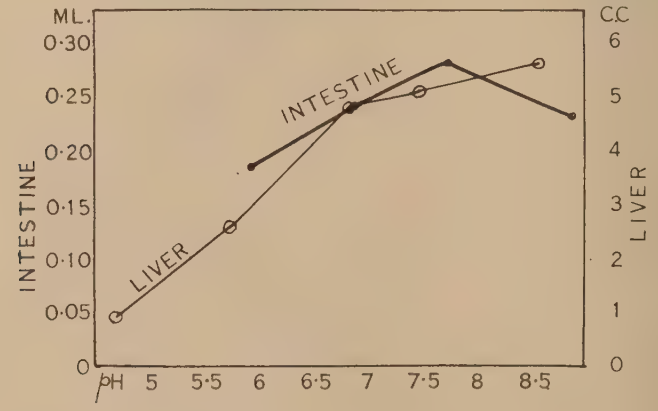

Fig. 5.—pH-Activity Curve of Lipases in Glycerol extracts of Intestinal Mucous Membrane and of Liver.

Substrate: triacetin. *Ordinates:* acid produced per hour by extract of whole organ from 1 kg. of fish in terms of N/5 NaOH.

● Expt. 61/63. Intestinal mucous membrane. Duration of digestion: 7 hours.

⊙ Expt. 94/95. Liver. Duration of digestion 2·5 hours.

The activity of the liver preparation is about 20 times as great as that of the intestinal preparation.

added to the tubes containing starch and the enzyme extract, and starch and the boiled extract, respectively; the absence of blue colour, or the

presence of a purple or red colour, indicates that some digestion has taken place. More precise studies were then undertaken in which the sugar concentration of the digests was estimated by Benedict's method. Since, in many cases, the volume of solution available was insufficient to reduce completely even 1 ml. of Benedict's solution, the titration was carried to completion with a standard solution of maltose, and the sugar concentration (in terms of maltose) of the digest estimated by difference.

RESULTS.

A. Stomach.

No amyloclastic activity could be detected in the stomach extracts at pH 2, 4 or 6.

B. Intestine.

A definite, but not very powerful activity was found in the extracts of the intestinal mucous membrane, with an optimum hydrogen-ion

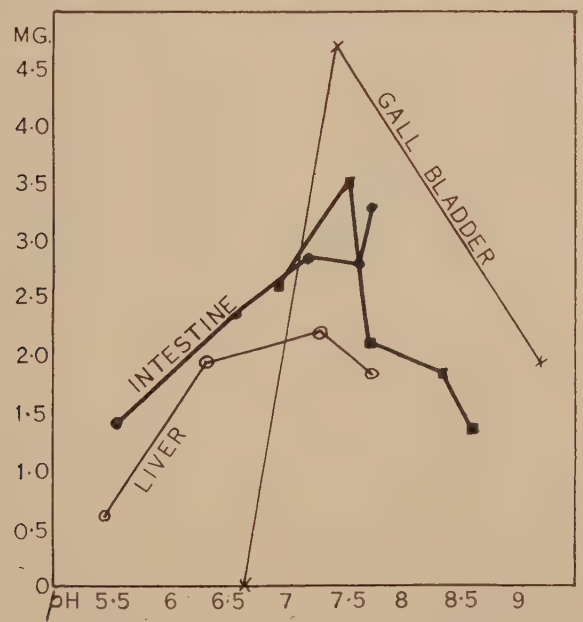

FIG. 6.—pH-Activity Curve of Amylases in Glycerol extracts of Intestinal Mucous Membrane, of Liver, and in Bile extract of Gall-Bladder.

Substrate: starch. *Ordinates:* mg. sugar (as maltose) produced per hour by extract of whole organ from 1 kg. of fish. Duration of digestion: 23 hours.

● Expt. 41. Intestinal mucous membrane.
■ Expt. 43. Intestinal mucous membrane.
⊙ Expt. 99/100. Liver.
× Expt. 99/100. Gall-bladder.

concentration at pH 7·5 to 8·0 (Fig. 6). The extracts of the portion distal to the cæca had a definitely greater action than those of the portion containing the cæca, so that it would appear that the enzyme is secreted by the cells of the intestine, and not primarily by those of the pancreatic tissue.

It was observed, somewhat unexpectedly, that the addition of small amounts of NaCl (0·05% to 0·1%) to the digests resulted in a marked increase in activity as shown in Table V. This has been observed by Cole

TABLE V.

Amyloclastic Action of Intestinal Extracts.

Solutions contained initially 0·3% starch, buffered at pH 7·5 and 1 ml. of extract. Sugar concentrations are expressed as mg. maltose per ml.

				Sugar Concentration after 24 hours at 25°.		
Expt. No.	Wt. of Fish g.	Portion of Intestine.	Total Volume of Extract.	Boiled Extract.	Active Extract.	Active Extract +0·07% NaCl.
38	520	Prox. 45 mm.	3 ml.	0	0·7	1·35
		Dist. 275 mm.	3 ml.	0	1·2	1·0
39	410	Prox. 12 mm.	5 ml.	0·35	0·65	1·0
		Dist. 170 mm.	6·5 ml.	0·45	0·8	1·45

(1903) in the case of human ptyalin, but it was not expected that the concentration of chlorides in extracts from marine fishes would be low enough for the effect to be observed. Actually, one extract from the proximal portion was found to contain only 0·0065% NaCl, two from the distal portions to contain 0·03% and 0·05% respectively, and one from the whole intestine 0·023%.

C. Bile.

No amyloclastic action could be detected in the bile unless it had been allowed to extract the gall-bladder. The activity was then comparable to that of the intestinal extracts, and had the same optimum pH (Fig. 6).

D. Liver.

Glycerol extracts of the liver had an amyloclastic action of the same order of intensity as those of the intestinal mucous membrane; the optimum pH appears also to be the same (Fig. 6).

The Hydrogen Ion Concentration of the Contents of the Alimentary Canal.

If the enzymes extracted from the mucosa of the alimentary canal are to be of any service to the animal, the medium in which they work must

be of the correct hydrogen ion concentration. Accordingly, in a number of fishes, the abdomen was opened, a ligature tied round the rectum, another round the pyloric sphincter and an artery clamp placed on the lower end of the œsophagus. The whole alimentary canal was then dissected out, the stomach and intestine slit open, and the hydrogen-ion concentration of the contents measured with the quinhydrone electrode. Bile was obtained by slitting the gall-bladder in some experiments, and by cannulation of the bile duct in others.

In the majority of fishes taken from the aquarium tanks, the stomach was found empty. In these cases, two drops of distilled water were placed on the mucous membrane of the opened stomach, and, a moment or two later, withdrawn into the electrode vessel, which held 0·05 ml. The intestine always contained a clear watery fluid with a certain amount of fæcal masses, unless, of course, it contained food remnants.

The results are presented in Table VI.

TABLE VI.

Acidity of Contents of Alimentary Canal and of Bile.

	Stomach.		Intestine.		Bile.
Fish No.	Contents.	pH	Contents.	pH	pH
7	Empty	2·7	Scraps of food	7·7	–
8	Empty	–	Traces of food	8·2	–
9	Empty	6·7	Traces of food	8·5	–
10	Empty	–	Traces of food	8·0	7·3
12	Empty	–	Traces of food	8·3	7·4
16	Polychætes	5·55	Fæcal matter	8·65	–
18	Not identified	–	Scraps of food	7·43	–
19	Crustacea—not identified	–	Traces of food	7·55	–
21	Empty	7·33	Traces of food	8·33	–
22	Empty	7·05	Traces of food	8·10	–
23	Empty	6·45	Empty	8·10	–
30	0·5 c.c. fluid	2·40	Traces of food	8·10	–
64	Not identified	6·45	Food—not identified	8·35	–
65	Not identified	6·30	Not identified	–	–
66	Full of clear fluid	6·85	Clear fluid only	8·20	–
75*	Empty	–	Traces of fæces	–	5·75‡
81*	0·5 c.c. fluid	5·60	Traces of fæces	–	7·60† 8·00‡
82	Empty	–	Traces of fæces	–	7·70 7·80†
83	Empty	+	Traces of fæces	–	7·25 7·35†
84	Empty	–	Traces of fæces	–	7·60 7·70†
88*	Clear fluid	6·7§	Clear fluid only.	–	–
89*	Clear fluid	–	Clear fluid only	–	8·05
90	4 c.c. clear fluid	3·90	Clear fluid only	–	7·60
99	Upogebia almost undigested. 5 drops of water added	7·60	Upogebia. Partly digested.	8·25	–
100	Upogebia almost completely digested	3·15	Upogebia almost completely digested	8·20	–

* Pilocarpine experiment.
† After dilution four-fold.
‡ After dilution four-fold and boiling.
§ By indicator.

It will be observed that in no case did the acidity of the food within the stomach reach so high a value as one would expect if the digestion were being carried out by pepsin. It must be remembered, however, that the figures given in the table represent the mean acidities of the whole food mass, and it is highly probable that on the surface, where digestion is taking place, the acidity would be very much greater.

In order to confirm the fact that the stomach is capable of secreting acid, a number of fishes were anæsthetised with urethane, and placed on an artificial circulation of sea-water, containing about 0·8% to 1·0% urethane. Aeration was carried out, and the circulation maintained, by a modification of the spray method described by von Euler and Heymans (1932). The concentration of urethane was adjusted so as to be high enough to prevent reflex activity, but not so high as to stop the respiratory movements entirely. The fishes appeared to survive well under these conditions, many experiments lasting over 5 hours. Since it was felt that it was undesirable to pass a stomach tube, owing to the risk of leakage of sea-water through the cardiac sphincter, a cannula was tied in the pyloric sphincter. All dissection, except the first skin incision, was done with an electric cautery, and not more than one or two drops of blood were lost.

The following experiments were performed :—

(1) In several fishes, 1 to 1·2 ml. of clear watery fluid of pH 5·5 to 5·9 were withdrawn as soon as the cannula was inserted.

(2) 1 ml. of sea-water (pH 8·3) containing phenol red was placed in the stomach, and withdrawn a few minutes later a full yellow colour (pH less than 7).

(3) 1 ml. M/10 Na_2CO_3 were inserted, withdrawn after 2 hours and titrated with N/10 HCl. It was found that 0·07 millimols of acid (equivalent to 0·7 ml. N/10 HCl) had been added while the solution was in the stomach.

(4) 1 ml. of M/10 phosphate buffer pH 8·0 was inserted and withdrawn after 1 hour with a pH of less than 7. 0·1 ml. of N/5 NaOH were required to bring it back to pH 8·0 ; this is equivalent to the secretion of 0·02 millimols of acid.

In an attempt to obtain a sample of bile without handling the gall-bladder, the common bile duct of one fish was cannulated, and 2 ml. of N/10 HCl inserted into the duodenum ; it was hoped that sufficient cholecystokinin might be liberated to cause a contraction of the gall-bladder. This anticipation was not realised, and the bile had to be expelled by pressure on the gall-bladder. (This bile contained no proteoclastic enzymes.) After half an hour, the intestine was removed and the contents titrated with N/5 NaOH. 1·5 ml. of N/10 HCl had disappeared, either by

neutralisation or absorption. It is impossible to be certain whether this indicates a true secretion of base by the walls of the intestine, or whether there had been merely a diffusion interchange with the buffers of the blood, but in any case there is no doubt that the acid secreted by the stomach can be neutralised in the intestine.

The Relative Potency of the Extracts from Various Organs.

The amount of digestion produced per hour by the whole organ from 1 kilogram of fish has been calculated for the proteases, lipases and amylases of the stomach, intestine, gall-bladder and liver. They are given in Table VII. Owing to the very great variation between different samples,

TABLE VII.

The Relative Potency of the Extracts from Various Organs.

Organ.	Number of Experiments.	Activity. Greatest.	Least.	Average.
A. Proteases. (1) Kerridge Method. Activities in mg. protein digested per hour per kg. fish				
Stomach. (*Glycerol extract*)				
more than 12 hours digestion	3	10·5	3·4	7·0
less than 12 hours digestion	9	54·5	5·2	25·8
Stomach. (*Acid extract*)				
less than 12 hours digestion	3	31	11·5	21·5
Intestine.				
more than 12 hours digestion	11	25	0	7·3
less than 12 hours digestion	2	19	14	16·5
(2) Formol Titration Method. Activities expressed as decrease in peptide linkages (ml. N/50 NaOH) per hour per kg. fish.				
Intestine				
more than 12 hours digestion	19	0·35	0	0·16
less than 12 hours digestion	4	2·5	0·46	1·25
Gall-Bladder				
more than 12 hours digestion	3	0·53	0·21	0·40
less than 12 hours digestion	3	10·0	3·2	5·9
Liver				
less than 12 hours digestion	5	5·2	2·2	3·4
B. Lipases. Activities expressed as amount of acid produced (as ml. N/5 NaOH) per hour per kg. fish.				
Intestine	7	0·92	0·12	0·35
Gall-Bladder	2	0·20	0	0·10
Liver	2	5·3	2·3	3·8
C. Amylases. Activities expressed as mg. sugar (as maltose) produced per hour per kg. fish.				
Intestine	6	9·0	2·8	5·0
Gall-Bladder	2	5·1	4·7	4·9
Liver	1	–	–	2·2

it has been felt advisable to include the highest and lowest values, as well as the average. In the case of the proteases, it is clear that the activity falls off very markedly in the first few hours, so that the activity figures

calculated from experiments of long duration are always lower than those calculated from experiments of short duration. In the table, a " long " experiment has been arbitrarily defined as one continuing for 12 hours or longer, and a " short " experiment as one continuing for less than 12 hours.

It may be concluded from these figures (*a*) that the proteoclastic activities of the gall-bladder and liver are approximately the same, and are greater than those of the intestine and stomach ; (*b*) that the lipoclastic activity of the liver is very much greater than that of either the gall-bladder or intestine ; and (*c*) that the amyloclastic activities of the intestine, gall-bladder and liver are all approximately the same. The stomach has no lipoclastic or amyloclastic activity.

SUMMARY.

1. It has been shown that the stomach of the plaice can digest proteins by an extra-cellular enzyme—apparently pepsin. Fats and carbohydrates appear to be unattacked in the stomach.

2. In the intestine, proteins are further attacked and the mucous membrane contains a trypsin and an erepsin.

3. The liver and the wall of the gall-bladder are more potent sources of trypsin than is the intestine ; the former contains, also, a cathepsin, and the latter an erepsin. It is not certain whether any of these enzymes can be secreted into the intestine ; administration of pilocarpine failed to make the bile definitely active in digesting proteins, but it is possible that there may be some other route by which the secretion may reach the intestine.

4. Fats and carbohydrates are digested in the intestine, since the mucous membrane of the intestine—and also the wall of the gall-bladder and the liver—contain lipase and amylase. The liver is a far more potent source of lipase than is either the gall-bladder or the intestine, but this lipase may be partly intra-cellular in action.

5. The hydrogen-ion concentration of the contents of the stomach and intestine has been shown to be consistent with the extra-cellular action of the enzymes found in the respective mucous membranes. The mucous membrane of the stomach is capable of secreting acid, and that of the intestine is capable of bringing about its neutralisation.

REFERENCES.

Anrep, G. V., Lush, J. L., and Palmer, M. G. 1925. Observations on pancreatic secretion. J. Physiol., **59**, 434.

Babkin, B. P., and Bowie, D. J. 1928. The digestive system and its function in *Fundulus heteroclitus*. Biol. Bull., **54**, 254.

COLE, S. W. 1903. Contributions to our knowledge of the action of enzymes. Part I. The influence of electrolytes on the action of amylolytic ferments. J. Physiol., **30**, 202.

COLE, F. J., and JOHNSTONE, J. 1901. L.M.B.C. Memoirs VIII. *Pleuronectes*. London.

DAWES, BEN. 1930. The absorption of fats and lipoids in the Plaice (*P. platessa* L.). J. Mar. Biol. Assn., **17**, 75.

EULER, U. S. v., and HEYMANS, C. 1932. An oxygenator for perfusion experiments. J. Physiol., **74**, 2*P*.

KERRIDGE, P. M. T. 1931. A new clinical method for the estimation of protein in urine. Lancet, **221**, 21.

MACKAY, M. E. 1929a. The digestive system of the eel-pout (*Zoarces anguillaris*). Biol. Bull., **56**, 8.

—— 1929b. Note on the bile in different fishes. *Ibid.*, **56**, 24.

NORTHROP, J. H. 1932. Pepsin activity units and methods for determining peptic activity. J. Gen. Physiol., **16**, 41.

STERN, K. G. 1931. Über die autolytische Wirksamkeit der tierischen Gewebsproteinasen und ihre Beeinflussung durch Schwermetalle. Biochem. Z., **234**, 116.

VONK, H. J. 1927. Die Verdauung bei den Fischen. Z. vergl. Physiol., **5**, 445.

WILLSTÄTER, R., and BAMANN, E. 1928. Über die Proteasen der Magenschleimhaut. Erste Abhandlung über die enzyme de Leukocyten. Z. physiol. Chem., **180**, 127.

Stolonization in Myrianida.

By

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With 6 Figures in the Text.

Myrianida pinnigera (Montagu) is one of the representative Syllids in the Plymouth district. Here, it is found always in close association with *Ascidiella aspersa* and *Phallusia mammillata*, possibly feeding upon the body fluid of these Ascidians.

The reproductive method of the Syllid has not been carefully studied. So far as observations were made during my stay at the Plymouth Laboratory from June, 1927, to the next June, those specimens collected from April to October almost constantly carried a more or less long chain of sexual buds, either male or female, while the others obtained in the winter, especially in January and February, were either without the chain of stolons or just at the beginning of its formation. *Myrianida pinnigera*, in the Plymouth district at least, appears to stolonate in the warmer half of the year, while the process stops in the winter time.

The number of setigerous segments in the resting stage varies from 50 to 82, with the maximum frequency at 65 (Table I).

TABLE I.

No. of segments counted	50	52	55	56	58	60	64	65	67	68	69	70	71	72	77	78	79	80	82
No. of individuals observed	1	1	1	3	1	2	3	5	3	3	2	4	1	1	1	2	1	3	1

In the budding specimens the number of segments which remained in the parent body is shown in the next table (Table II).

TABLE II.

No. of segments in the stock	34	37	40	44	48	52	56	60	64	67	70	73	76
No. of individuals	1	1	4	7	6	8	12	8	5	2	3	1	1

From the data in the first table we see that 50 is the smallest and 82 the largest number of segments for the resting stage. From the data in the second table we find the most anterior position of budding between segments 34 and 35 and the most posterior position between segments 76

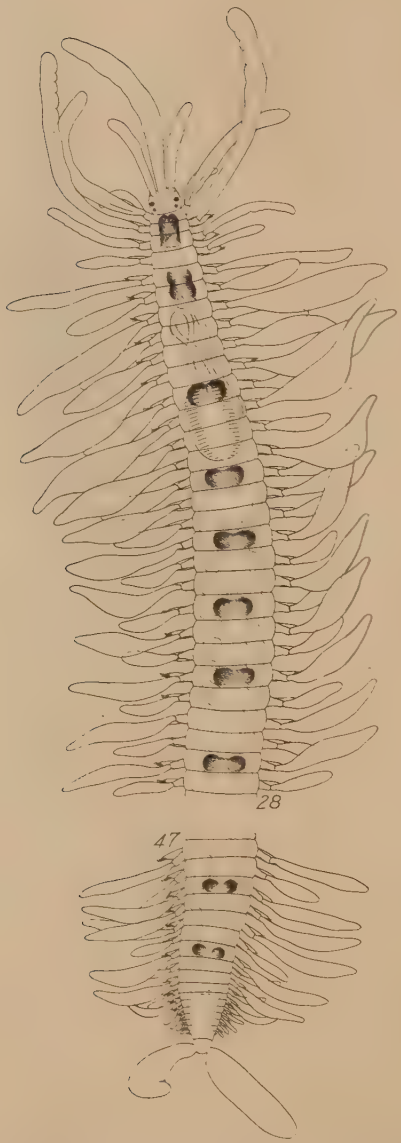


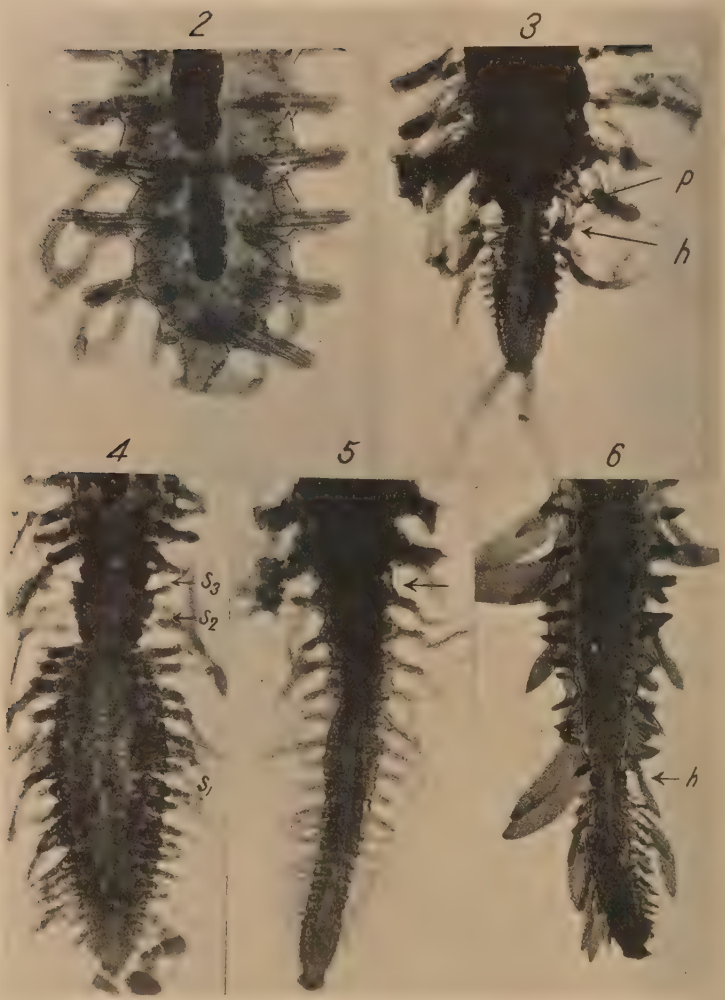
Fig. 1.—*Myrianida pinnigera.* Non-proliferating phase.

and 77. There is therefore no reason to believe that the stolonization of the present species takes place always at the posterior end of the 66th setigerous segment as A. Malaquin (1893) has stated in his monograph (p. 289).

If we draw two graphs on the same horizontal plane, one for the segmental variation of the resting stage and the other for that of the parent body in the budding individuals, in such a way that the number of worms examined is plotted against the number of segments counted, the first part of the curve of stolonization evidently overlaps the posterior part of the curve that represents the segments of the resting stage. This fact at once reminds us that in the stolonization of Myrianida, as in most species of Autolytus (gemmiparous forms), there is, at least at the beginning of a chain formation of stolons, the process of simple schizogamy producing a single stolon by the separation of the posterior segments from the anterior ones ; first of all there appears a new embryonic segment between two old ones at a certain distance from the posterior end at the point of separation. Actual observation shows that this distance is more than 16 but less than 22 segments. But in no case does the schizogamous stolon separate from the parent body before the appearance of the second, third and more stolons of higher order. Therefore schizogamy in the strict sense, forming a single stolon, in this case exists only temporarily.

Malaquin (*l.c.*, p. 312) describes in his case 3 of schizogamy in *Autolytus Edwarsi* a number of newly formed segments already intervening between the head of the stolon and the posterior end of the parent body. In case 4 he figures a stage long after the detachment of the first stolon, when gemmiparous proliferation has become well established, the embryonic segment being followed by a chain of 4 stolons in gradually increasing development. It is supposed that the process of stolonization in Myrianida is simply a combination of these two cases, case 3 and case 4 of *Autolytus Edwarsi*, and Malaquin (*l.c.*, p. 314) has actually described such in case 7, where a parent body of 28 segments is followed by a chain of sexual individuals which is produced by gemmation succeeding schizogamy, passing from case 3 to case 4 above mentioned. In Myrianida such a transition from schizogamy to gemmiparity takes place at the very beginning of stolonization ; that is to say before the separation of the first schizogamous stolon a number of secondary ones are quickly produced from the embryonic segment by successive stolonization.

It is true that some specimens are observed carrying a chain of stolons in such anterior positions as the 34th and 37th setigerous segments, while others carry it in such posterior positions as the 73rd and 76th segments. Nevertheless, we need not necessarily suppose here a forward movement of the position of stolonization such as Malaquin has claimed for the origin



FIGS. 2–6.—Regeneration and stolon-formation in Myrianida.

Abbreviated terms used in the figures : *h*, head of stolon ; *p*, zone of proliferation ; S_1, S_2, S_3, stolons of 1st, 2nd and 3rd order ; arrow in Fig. 5 indicating the original position of cut.

of those specimens of *Autolytus Edwarsi* which carry the chain of stolons in an anterior position, as for example between segments 25 and 30 (most often on 27 or 28), since there are in *Myrianida pinnigera* individuals in the resting generation possessing only 50 segments.

It is most interesting to notice that the positions of separation, which show so wide a range of variation, are never continuous even in two consecutive segments. On the contrary, they are entirely discontinuous. But in spite of that the position is almost always fixed after the 34th setigerous segment at one of the following segments : 34, 37, 40, 44, 48, 52, 56, 60, 64, 67, 70, 73, 76 and so on. To find the reason for this let us return to a former paper (Okada, 1929, p. 587) and see the rule of fragmentation of the Syllid which is represented by the formula H13–3–3–3–4–4–4–3–3–4–4–4–4–4–4–3–3–3–3– - - - - - - - - - - - - - - xp. In accordance with this formula the segmental arrangement of *Myrianida pinnigera* after the 34th setigerous segment can be divided into the following groups : segments 34–37, segments 38–40, segments 41–44, segments 45–48, segments 49–52, segments 53–56, segments 57–60, segments 61–64, segments 65–67, segments 68–70, segments 71–73, segments 74–76 and so on. A further characteristic point is that the most anterior segment of each group bears a special pigment pattern on the median dorsal surface (see Fig. 1), and in the range of stolonization which covers the median part of the body from behind about the 30th setigerous segment to about the 20th segment from the posterior extremity, the formation of a chain of stolons takes place always at the posterior boundary of each segmental group.

In the part of the body just mentioned, if a worm in the process of budding is cut at a given position and the longer posterior part is removed, including the chain of stolons, the missing segments are able at once to regenerate and after a certain while they directly develop into a new chain of stolons, leaving the most anterior segment for the source of the further growth. The posterior cut end of the parent body remains as before. Therefore, the positions of stolonization are by no means absolutely restricted to certain segments and can be experimentally altered to any segment within the range of stolonization mentioned above. Figures 2–6 show 3 developmental stages of the posterior segments experimentally induced to regenerate from a segment which in the normal condition does not constitute a position of separation. Figure 2 represents a still very young stage before indication of the stolon formation. In Figure 3 the first stolon is about to be produced through direct metamorphosis of the regenerated segments leaving one or two at the proximal side for the future proliferation of new segments. Thus in Figure 4 already 2 stolons beside the first one are produced.

On the other hand if the same cut is done outside the range of stoloniza-

tion, i.e. in front of some 30th setigerous segment, for example, there is an easy regeneration of the posterior segments as in the preceding case, but there is no immediate formation of stolons (Fig. 5).

In Figure 6 the beginning of natural stolonization in *Myrianida pinnigera* is reproduced from a microphotograph taken at the Plymouth Laboratory. Here schizogamy, with the production of a single stolon, is distinctly shown.

REFERENCES.

Okada, Yô K. 1929. Regeneration and Fragmentation in the Syllidian Polychætes. Arch. f. Ent.-Mech., 115.

Malaquin, A. 1893. Recherches sur les Syllidiens. Mém. soc. sci. Art., Lille.

A Note on the Feeding Habits of *Chimaera monstrosa*.

By

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With 2 Figures in the Text.

Bashford Dean in his Monograph on the Chimaeroid Fishes (1906), describing the North Pacific species *Chimaera colliei*, states that it is omnivorous and that the " broken shells of mollusks are commonly found, as well as fragments of good sized crustaceans, as indeed the scanty literature records." He further states that " in the gut of *C. monstrosa* Faber finds crustacean and shell-fish fragments; Monticelli, quoting Lütken, *Cyprina islandica*; . . . Olsson finds also (and his observations are the most detailed hitherto published on the feeding of Chimaera) chætopods, amphipods, echinoids and polyps."

The following is an account of the gut contents of several specimens of *Chimaera monstrosa* which the authors were fortunate enough to obtain recently.

The specimens which were taken from the Atlantic Ocean sixty miles N.W. of Black Rock (Lat. 54° N., Long. 12° W.) at a depth of 220 to 250 fathoms, were preserved in dilute formalin.

The actual examination of the gut contents was carried out as follows.

The œsophagus and rectum were ligatured, and that part of the gut lying between the two ligatures was removed and placed in a dish of dilute formalin. The gut was then opened by a longitudinal incision, and its contents carefully transferred to the dish where they were subsequently examined by means of a binocular microscope. The food fragments were then provisionally classified, care being taken not to separate such parts as may have belonged to the same organism during life. A detailed examination of the fragments was carried out later, but because of the close similarity of the gut contents of various specimens, only three were examined in detail.

Food found in the gut of the three specimens.

Fragments of	Ophiuroids.	Ossicles, spines, etc.
,,	Crustacea.	Mostly small Malacostraca, e.g. Crabs and Galatheids. Isopods were present.
,,	Fishes.	Muscle and cycloid scales.
,,	Annelids.	Aphrodite.

The remains of Ophiuroids were particularly abundant in all three specimens. Whilst parts of Crustacea were present in all three, they were only abundant in two specimens. Only one specimen contained unmistakable Annelid remains; a solitary, but almost complete specimen of Aphrodite. In addition, Gyrocotyle and Sporozoans were found as gut-parasites in all the Chimaeras examined.

Discussion.

It is noteworthy that no mollusc remains were found in any of the specimens that we examined. This is possibly explained by the fact that

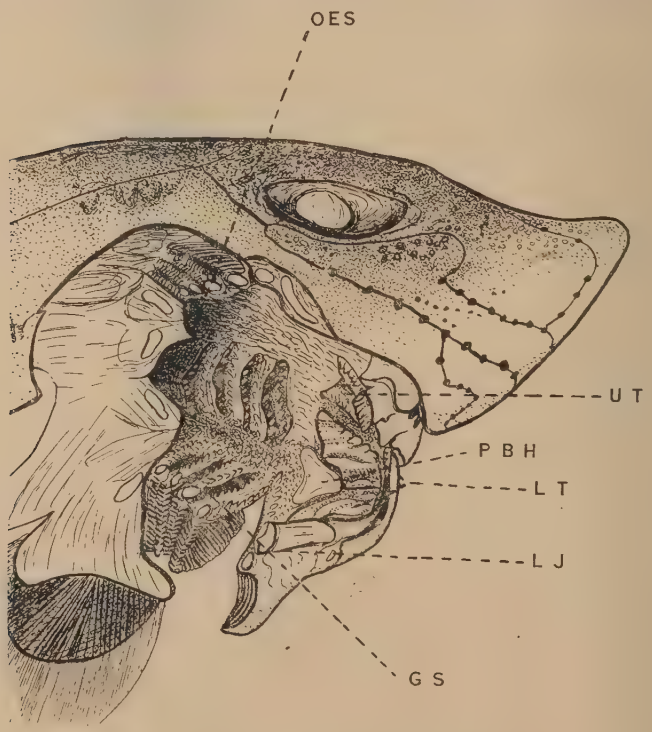

Fig. 1.—Dissection of Buccal Cavity and Pharynx.
GS, Gill-slits; LJ, Lower-jaw; LT, Lower-teeth; OES, Oesophagus; PBH, Pad on basi-hyal; UT, Upper teeth.

our fishes were caught in relatively deeper waters than those mentioned by other observers. (Bashford Dean mentions that *C. colliei* swims fairly near the surface.)

From the facts obtained from examination of the gut contents and of

the nature of the buccal cavity, it is possible to make certain inferences as to the feeding habits of the fishes.

The food-particles found in the gut are of irregular shape but never exceed three-quarters of an inch in length. In no case were recognisable portions of the " disc " of the Ophiuroids present despite the abundance of small portions of the " arms." This suggests that they had been bitten off from the " disc." From the condition of the more complete fragments of Crustacea (e.g. Crabs), too, it would appear as though the fish had seized them and bitten off certain parts (limbs) quite cleanly. Again, the presence of quite large cycloid scales and fish muscle but the complete absence of

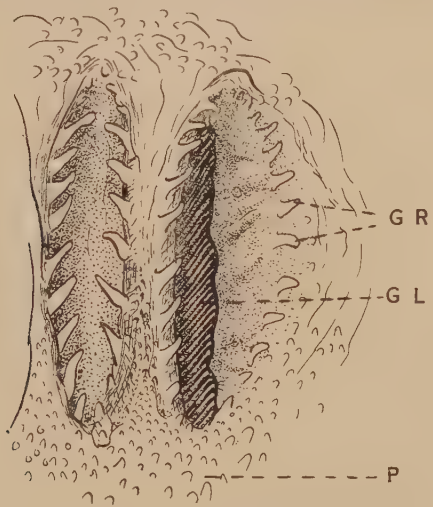

Fig. 2.—Gill Apertures 1 and 2.
GL, Gill lamella; GR, Gill-rakers; P, Papillæ.

anything in the nature of endoskeletal parts suggests that pieces had been bitten out of the prey, possibly whilst it was still alive.

In contrast to most carnivorous fishes which swallow their prey almost whole, it seems that Chimaera bites up its food into small fragments.

To this end the teeth are admirably adapted; the sharp edges alone of the teeth of upper and lower jaws meet when the mouth closes and would suffice to shear off portions of the food. It is probable that the hard pad of tissue over the basi-hyal serves to crush food against the plate-like teeth of the upper jaw. The small size of the food swallowed may possibly be accounted for by the fact that the autostylic nature of the skull and the shortness of the jaws restricts the gape of the mouth (Fig. 1).

Food particles are prevented from entering the pharyngeal apertures of

the gills by a system of gill-rakers. These are similar to, but larger than, the papillæ on the mucous membrane lining the bucco-pharyngeal region with which they are perhaps comparable (Fig. 2). Evidence that the gill-rakers are indeed modified papillæ was afforded by a microscopic examination of serial sections. The histology of both gill-rakers and papillæ was identical with the exception that the former were supported by an axis of cartilage which was not, however, connected to the branchial arch below it. In both cases, the outer layer (mucous membrane) was perforated by a system of fine canals, the structure of which is being further investigated.

REFERENCE.

Bashford Dean. 1906. Chimaeroid Fishes and their Development. Carnegie Institution of Washington, pp. 1–172.

A Case of Hermaphroditism and Viviparity in *Echinocardium cordatum.*

By

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With 4 Figures in the Text.

In May, 1933, during routine gonad examinations, a hermaphrodite specimen of *Echinocardium cordatum* was observed. It was collected in the sandy beach of Port Erin Bay at low water of ordinary spring tides, and was 4·7 cm. in length, which corresponds, in a normal specimen, to an age of about four years. The external colour was dark, although not more so than in other specimens in the batch, but the gonad was exceptionally pigmented—almost black. In this locality the colour of the test and gonad becomes progressively darker with increasing age, and affords some measure of the latter.

The specimen was examined within half an hour of being collected, and portions of the gonad were fixed within ten minutes of being opened, so that fertilization and segmentation could not have advanced in the laboratory to the stage found. Smears from the gonad showed ova, of which about 50% were ripe, together with ripe spermatozoa. The gonoduct contained ripe ova, together with segmenting ova and early embryos. Figure 1 is a photograph of the contents of the gonoduct, taken immediately after opening, and showing ova with a four-cell stage and a blastula. Cultures were set up in sterile sea-water, from the contents of the gonoduct, and also from the gonad itself, as well as a control fertilization from normal urchins, and from all of these normal early plutei were reared. Those cultures from the hermaphrodite specimen did not, however, develop much further, possibly owing to premature fertilization in the abnormal conditions in the gonad.

Figure 2 shows a longitudinal section of a portion of the gonoduct containing a blastula as well as mature ova. No segmenting ova were seen actually in the follicles of the gonads (Fig. 3), but there were present a number of multinucleate bodies, about the size of an ovum, in which the protoplasm was partly broken down (Fig. 4), and no cell walls were visible. In more advanced stages the nuclei also seemed to have partially disintegrated. These bodies appeared to be fertilized ova in which

segmentation, or at least nuclear division had taken place, but which had failed to continue their development in a normal manner, and had begun

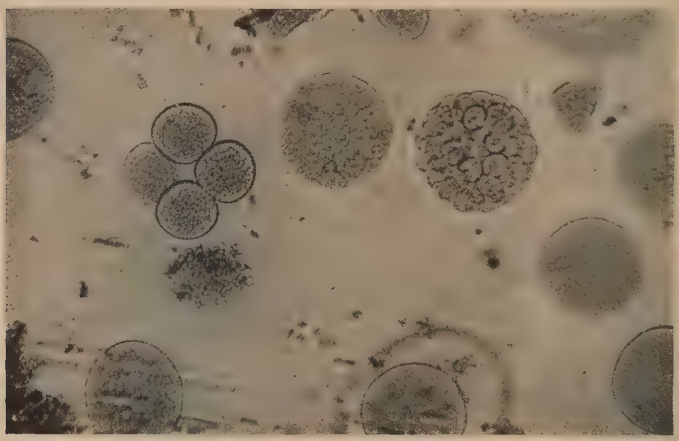

FIG. 1.—Ova and larvæ in gonoduct. × 100.

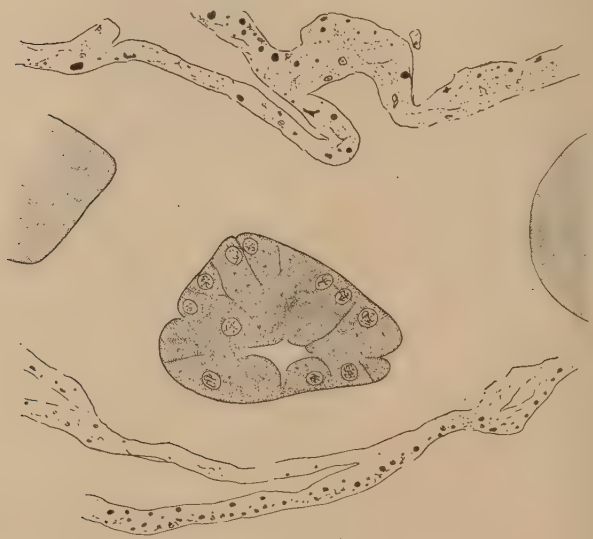

FIG. 2.—L.S. of gonoduct showing blastula. × 400.

to dedifferentiate. Those larvæ which had reached the gonoduct appeared to be developing more normally.

Apart from the viviparity of this specimen, it is of interest because of

its hermaphroditism, as comparatively few cases have been described in the Echinoidea, and, so far as the author is aware, only one in the Spatangoidea. It is, however, a normal condition in some other echinoderms.

In *Asterina gibbosa* the condition with regard to hermaphroditism

FIG. 3.—T.S. of gonad follicle showing male and female portions. × 750.

varies according to the locality (Cuénot, 1898). At Roscoff, during the first one or two years of its life, it is male, although sperm are shed only once; the starfish then becomes and remains female. At Banyuls they

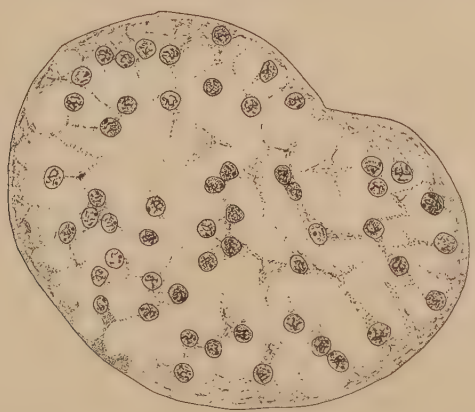

FIG. 4.—T.S. of degenerating embryo in gonad. × 100.

are male for a longer period, shedding sperm for several successive years, and then becoming completely female. At Naples there is no fixed relation between size and sex, and males, females and hermaphrodite individuals may be found at all sizes. Cuénot says further that, although

individuals do not normally function as both male and female at the same time, he has found such specimens, with both ova and sperm ripe at the same time, at Banyuls and at Naples.

In the Echinoidea, hermaphroditism is recorded as occurring in several species. Heilbrunn (1929) describes a specimen of *Arbacia pustulosa* with four of the segments of the gonad alternately male and female, and the fifth an ovo-testis with mixed normal male and female tissue. A self-fertilization yielding normal larvæ was obtained. Gray (1921) describes another specimen of the same species in which four segments of the gonad had the normal female colouration, but proved, on examination, to be degenerate male tissue, while the fifth segment, mottled in appearance, contained the latter mixed with normal male tissue. He records also a specimen of *Paracentrotus lividus* with three segments of the gonad completely female, and the other two mixed female and male, both being ripe and fertilizable *inter se*. He does not state whether, as a result, the specimen was viviparous. Herlant (1918) also records a hermaphrodite individual of this species with three gonad segments normal male, one atrophied male, and one mixed male and female. He states that in the latter the male and female portions were mostly separate, but that in places they graded distinctly into one another, and showed oögenesis and spermatogenesis taking place side by side. He states further that the ova and sperm were ripe in the mixed gonad, and that they fertilized normally when mixed in sea-water, so presumably no fertilization had taken place within the follicles of the gonad. Drzewina and Bohn (1924) record another individual of this species from Roscoff, in which four segments of the gonad were male, and one female. Fertilization from these yielded healthy larvæ, which, however, began to develop abnormally in the pluteus stage. They also mention, but do not describe, a hermaphrodite *Echinocardium cordatum*.

Gadd (1906) records a hermaphrodite specimen of *Strongylocentrotus dröbachiensis* with one segment of the gonad male and the rest female. These were all ripe and fertilizable *inter se*. Viguire (1900) records a hermaphrodite individual of *Sphærechinus granulosus* but says little about it beyond the fact that it was the only one he had ever seen, and was self-fertilizable.

Hermaphrodite specimens of *Echinus esculentus* have been found occasionally. One of these (Moore, 1932) had three segments of the gonad ripe female, one unripe female, and the fifth ripe male. It also was self-fertilizable, and gave larvæ which developed into normal plutei. In most of these described cases the male and female portions of the gonad have been restricted to separate segments, and in none is it recorded that fertilization was taking place within the gonad.

Giard (1900) states that at Wimereux, *Echinocardium cordatum* is

normally a protandrous hermaphrodite, "ova beginning to appear about mid-July in gonads which have, up to then, been clearly male." Such a condition certainly does not hold at Port Erin, nor has the author been able to find it elsewhere in Britain. Of 358 specimens from Port Erin which were examined at a season when their sex could be determined from a smear of the gonad, 181 were males and 177 females. These were all animals in their third or subsequent season. At Port Erin, spawning does not occur in the first year. In second season urchins of the 1932 group, examined in January and February, 1934, when their gonads were maturing for the first time, a similar equal distribution of the sexes was found. And the hermaphrodite specimen described here was the only one which was found among several hundred examined. It would therefore be interesting if Giard's statement could be confirmed elsewhere.

BIBLIOGRAPHY.

Cuénot, L. 1898. Notes sur les Echinodermes. III. L'hermaphroditisme protandrique d' *Asterina gibbosa* Penn. et ses variations suivant les localités. Zool. Anz., Bd. XXI, p. 273.

Drzewina, A., and Bohn, G. 1924. Un nouveau cas d'hermaphroditisme chez l'oursin *Strongylocentrotus lividus*. Comptes Rendus Acad. Sci. Paris, T. 178, p. 662.

Gadd, G. 1907. Ein Fall von Hermaphroditismus bei dem *Strongylocentrotus drœbachiensis* O. F. Müll. Zool. Anz., Bd. XXXI, p. 635.

Giard, A. 1900. À propos de la parthénogénèse artificielle des œufs d'echinodermes. Comptes rendus Soc. de Biol. Paris, p. 761.

Gray, J. 1921. Note on true and apparent hermaphroditism in sea-urchins. Proc. Camb. Phil. Soc., Vol. XX, Pt. 4, p. 481.

Heilbrunn, L. V. 1929. Hermaphroditism in *Arbacia*. Science, New York, Vol. 69, 1790, p. 427.

Herlant, M. 1918. Un cas d'hermaphroditisme complet et fonctionnel chez *Paracentrotus lividus*. Arch. de Zool. Expér. et Gén., T. 57, Notes et Revue, p. 28.

Moore, H. B. 1932. A hermaphrodite sea-urchin. Nature, July 9, Vol. CXXX, p. 59.

Viguire, C. 1900. L'hermaphroditisme et la parthénogénèse chez les echinodermes. Comptes rendus Acad. Sci. Paris, T. 131, p. 63.

A Comparison of the Biology of *Echinus esculentus* in Different Habitats. Part II.

By

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With 10 Figures in the Text.

Echinus esculentus has been studied from the " Breakwater " ground on the side of the ruined breakwater in Port Erin Bay, from a depth of about one metre up to low water of ordinary spring tides, also from the " Breast " ground off Port Erin in 25 to 35 metres, and from the " Chickens " ground further offshore in 60 to 70 metres. An account of the seasonal changes in the urchin gonads from these grounds was given in a previous paper (Moore, 1934), where the grounds are described in fuller detail. Further samples were trawled on a sandy bottom in " Niarbyl " Bay near Port Erin in about 7 metres, and also, through the courtesy of Mr. R. Elmhirst, off Keppel Pier in the Clyde in about 9 metres.

Methods.

The method of collecting varies of necessity on the different grounds, and was in some cases selective with regard to size of urchin captured. On the Breakwater the samples were collected by hand, and a fair sample of the population was obtained. Some of the Breast samples were dredged, which yielded small but representative samples, while others were taken with an otter trawl which was liable to miss the smallest sizes. The two methods combined gave plenty of large and small urchins, but rather small numbers of intermediate sizes. The Keppel and Niarbyl samples were collected with otter trawls, and were deficient in the smallest sizes. The Chickens urchins were obtained from pots set by local fishermen for *Buccinum undatum*. Very small specimens were liable to be washed out of the pots during hauling if the weather was stormy, especially in winter. They were also apt to be overlooked by the fishermen. On the whole, however, the Chickens sample was representative.

Diameter and *Height* were measured with sliding calipers which were read to the nearest millimetre. The caliper ends were pointed for reading diameters, while for heights, one end was fitted with a knife-edge to span the peristome.

External volume was measured by displacement of water from a vessel fitted with an overflow pipe, or, in the case of dried shell, by displacement of lead shot from a vessel of known volume, and the results obtained were accurate within about $\pm 2\%$. *Volume alive* was measured in the same way. *Internal volume* was measured by filling the dried shell with lead shot, or, in the case of very small specimens, with mercury. The internal volume is related to the volume alive, with spines, by the formula $V_a = 1{\cdot}28 \times V_i$.

Local Variation.

One characteristic distinguishing the Breakwater from the Breast race has already been described, namely, the difference in volumes of their gonads, which, when full, reach nearly five times the size on the Breakwater that they do on the Breast. Those from the Chickens agree in this particular with the Breast (Moore, 1934). It appears that this difference is correlated with the amount of food available on the different grounds.

The difference in the growth rates of the urchins is discussed later. A further difference lies in the colour of the test. On the Breakwater this varies more or less uniformly between violet or mauve and red or yellowish. On the Breast the violet or mauve type is much rarer, although a few such specimens are to be found, while on the Chickens only a single mauve specimen was found among about a thousand urchins examined, the rest all being red. This test colouration must be distinguished from that of the spines which does not necessarily agree with that of the test, and appears to be due to an entirely different pigment. It is hoped in a later paper to discuss the nature of these pigments in more detail.

For a study of the local differences in test structure, samples of 150 to 170 urchins were taken from each of the three grounds, Breakwater, Breast and Chickens, and the following measurements made on each specimen—test diameter, test height, polar circumference, internal volume, external volume and test thickness. The latter was measured on the cut edge of one of the large interambulacral plates, which were found to give consistent results for any one specimen. It was hoped that polar circumference would prove to be a good measure of the size of the animal, not seriously varying with the shape of the urchin, but this was found not to be the case. In practice, therefore, either the internal volume of the test or its cube root was taken as the best measure of the size of the animal. Owing to the change in shape during the life of the urchin, and also to the racial differences in shape referred to below, diameter is not a very satisfactory measure of size, and is avoided wherever possible.*

The data for the shell thickness are shown in Figure 1, plotted against cube root of volume. The increase of thickness is not quite linear throughout life, the older animals being relatively slightly thinner shelled. The

* Conversion tables for diameter-volume are given in Table I.

Breast and Chickens races agree substantially, but differ from the Breakwater race in being about twenty per cent thinner. This difference is so marked that the local fishermen consider them to be two different kinds of urchin.

Figure 2 shows the variations in shape on the three grounds, together with the Niarbyl sample. There is a steady increase in relative height

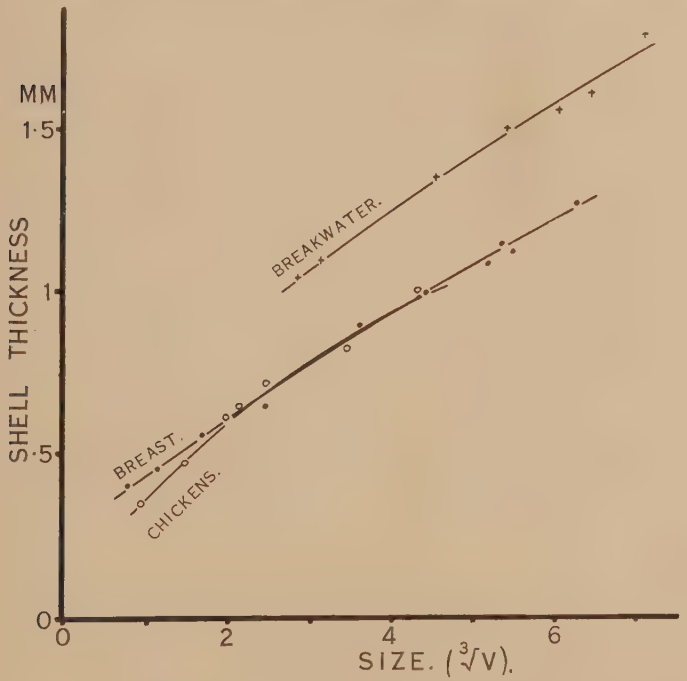

Fig. 1.—Relation of shell thickness to size.

throughout life on all grounds, but tending to become more or less constant in the largest individuals. The Breakwater, Breast and Chickens races differ slightly among themselves, but all range from a value of $\frac{\text{height}}{\text{diameter}}$ of about 0·5 when very small to about 0·7 when adult. The Niarbyl sample was strikingly tall throughout. Such very tall races are known to occur locally elsewhere. Elmhirst states (personal communication) that they occur on the Skelmorlie Bank in the Clyde, in about 12 metres.

D'Arcy Thompson (1917, p. 661 *et seq.*), in discussing the shape of urchins, shows that the downward and outward pull of the tube-feet will

tend to flatten the shell unless it grows thicker to resist this pull. The urchins from the Breakwater, being in a zone in which they are exposed to wave action, will have to exercise a greater pull with their tube-feet in order to resist dislodgment, than will similar urchins from the comparatively quiet waters of the Breast or Chickens grounds. Theoretically, therefore, the Breakwater urchins should either be flatter or else thicker shelled than those from the off-shore grounds, and in practice they are found to be both thicker and flatter. This seems to present at least a

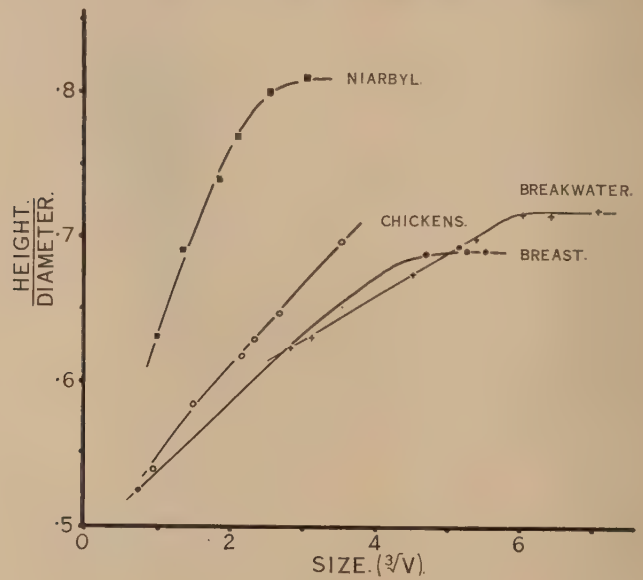

FIG. 2.—Relation of shape $\left(\frac{\text{height}}{\text{diameter}}\right)$ to size.

partial explanation of the differences, although diet probably plays a part also in determining thickness of shell, and the changes with age still remain to be explained.

SIZE DISTRIBUTION.

The Chickens ground, although atypical in the small growth rate found there, yields the simplest size data. The sampling on it is representative, and there is little likelihood of immigration of urchins from elsewhere on to this deep-water ground since it is bounded on the outside by a deeper area with unsuitable mud bottom. And the only known case of migration is in a shorewards direction on to the Breakwater.

About nine hundred Chickens urchins were measured, comprising

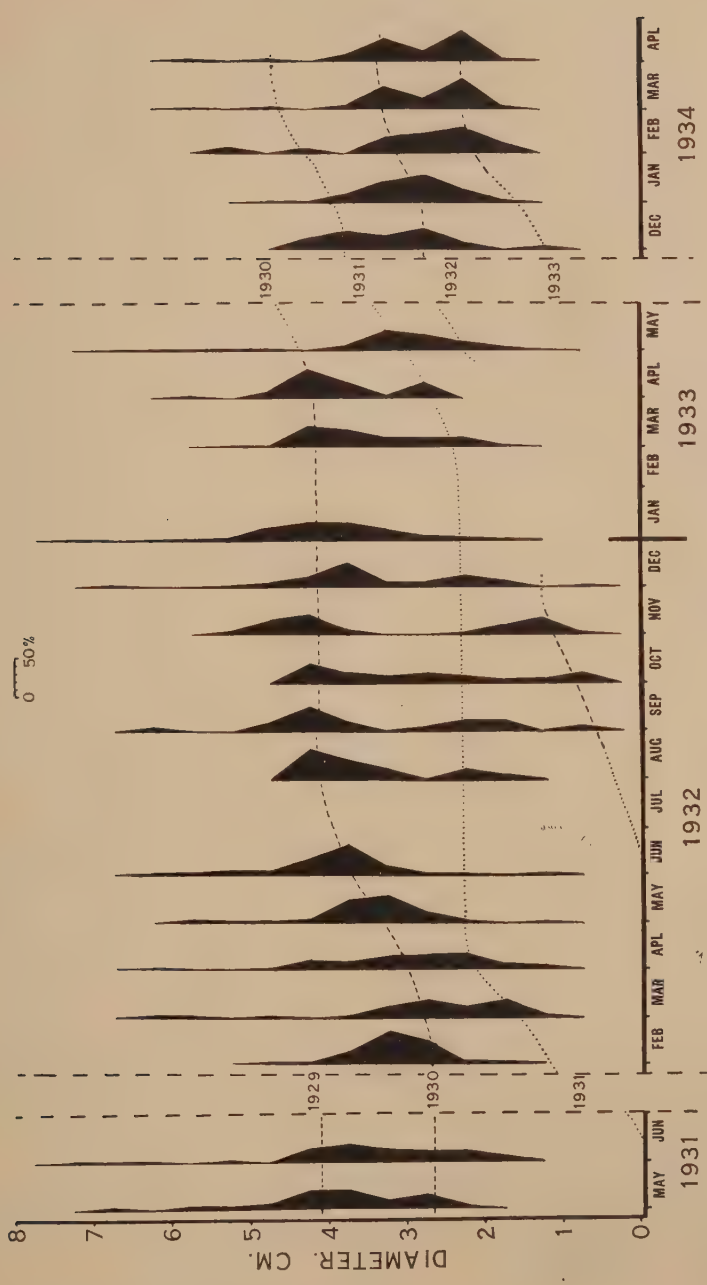

Fig. 3.—Seasonal changes in size distribution on the Chickens ground.

practically continuous samples from February, 1932, to May, 1933. The results are given in Table II, and as percentages in each five millimetre group for each month in Figure 3. As the groups are somewhat difficult to interpret correctly, a series of free-hand curves has been drawn to them, representing the growth of the average specimen of each year group. The growth of the 1931 brood is indicated with a more lightly dotted line as it was a bad year for urchins, and was represented in subsequent samples by too small numbers to be sharply defined. The 1932 brood, which should theoretically have settled out of the plankton about June, was first obtained in September, having presumably been too small to capture before that. In the winter months, owing to rough weather, the smaller sizes tend to wash out of the pots when these are being hauled, with the result that there is a gap in the curve for the 1932 brood between December and May, but thereafter they appear again in good numbers. The period of shell growth is very short, lasting only for about four months in the spring, after which, except in the first year urchins, there is almost complete cessation of growth for the rest of the year, as will be seen in Figure 3. The young, after metamorphosis, which takes place about June, grow continuously until their first resting period a year later. The March–July growth period corresponds to a time of rising sea temperature (see Table III), ceasing before the maximum temperature is reached, and not recommencing later in the year when it drops to the same level again. The spawning period—April and May—also falls within the growing period. The gonads of the male urchin make the whole of their year's growth in this March–July period, but those of the female, at any rate on the Breakwater, continue to grow until spawning again sets in (Moore, 1934).

The size attained in the June–February non-growing phase affords a measure for the comparison of growth in different years. At this time in 1932–33, the 1931 year group had a mean diameter of about 2·3 cm., while the 1930 and 1932 groups, at a corresponding age had diameters of 2·7 and 2·8 cm. respectively. 1931 was thus a very bad year for this species, and 1930 and 1932 normal years, 1933 appears to have been a very good year judging by the numbers of surviving members of that year group in 1934, but samples were not obtained sufficiently late in that year to determine the resting size for the 1933 group. The deficiency of the 1931 group is still seen in 1933–34, when their size is 3·8 cm., as compared with 4·2 cm., for the 1929 and 1930 groups at a corresponding age.

The variation in size attained by the different year groups might be accounted for either by direct variation in the available food supply during the year in question, or else by variation in time of hatching, and hence in time available for growth before the onset of winter. Probably both contribute to the observed effect. The favourability of a given year

appears to be correlated with the sea temperature prevailing. MacBride (1914, p. 517) gives the larval life of *Echinus esculentus* in captivity as forty-five to sixty days, and this has been confirmed at Port Erin. Taking this figure, the larval planktonic life of the urchin will be during the period March–June. The weekly sea temperatures at Port Erin during this period had a mean deviation from the normal (obtained from the analysis of 25 years results) of —0·69° C. in 1931, +0·13° in 1932 and +0·24° in 1933. The rate of rise of sea temperature at this period is 4·8° C. per hundred days, so that the above temperatures may be translated into a retardation of fourteen days in 1931, and accelerations of three and five days in 1932 and 1933 respectively. The difference in time of 50% spawning in 1931 and 1932 was in the same direction, and of the order of eleven days.* The unfavourableness of 1931 at Port Erin was reflected also in the very small spat-fall of *Echinocardium cordatum*, while 1932 and 1933 were good years for that species, and 1933 was also noticeable for an exceptionally heavy spat-fall of *Balanus balanoides*.

The size distribution data from the Breakwater are of a more complicated nature. In the first place the population on this ground is supplied entirely by migration from deeper water, and no young specimens at all are to be found on it. With the omission of at least one complete year group, the only available method of dating the several peaks found in size distribution curves from this locality is on the evidence of annual rings, as is described later. The second difficulty arises from the presence of double peaks for each expected year group. This was found in a large sample of 276 urchins in November–December, 1933, and confirmed in a further sample of 149 the following February (Table IV). In a further sample of 160 urchins in March, 1934, the sexes were therefore distinguished, and the peaks now resolved themselves into a simple series, with the females in each case slightly larger than the corresponding males, as shown below (and in Table V).

Breakwater. Year group sizes, March, 1934. Diameters in cm.

♂	–	6·55	(7·7)	8·4	–	–
♀	4·8	6·8	8·0	8·65	9·6	10·6

The records from the earlier work on the gonads of this species provided further seasonal size distribution data in which the sex was recorded for each animal. The results for six hundred and fifty-four urchins are given in Tables VI and VII, and curves drawn from these in Figure 4. These show a summer–autumn period when there is no growth, similar to that found on the Chickens, but here the period of growth is of longer duration. The two sexes, while finally attaining the same size each year, have their period of growth at a slightly different time, and the interesting point

* See Table III for Port Erin temperature data.

emerges that in the cold year 1931 it was the males which grew before the females, while in the warm years 1932 and 1933 the females grew before the males. The difference in growth period on the Breakwater and Chickens grounds probably reflects the very different conditions of food in the two localities.

Annual Rings and their Evidence on Growth Rate.

At the suggestion of Dr. A. C. Stephen, an attempt was made to find some structure in the urchin which showed bands comparable with the annual rings in many fish vertebræ, and otoliths, mollusc shells, etc. The

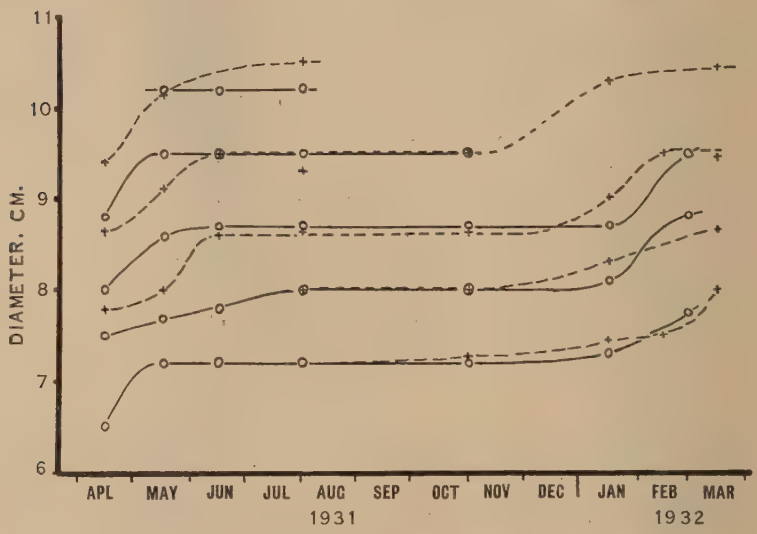

Fig. 4.—Growth of successive year groups on theBreakwater. ——— ♂; - - - - - ♀.

following method, although laborious, proved the most satisfactory, but it should be noted that any of the other plates of the urchins might have been used. The apical system of the urchin was removed with scissors, and air dried. In larger specimens the genital plates were then separated and treated separately, while in urchins which were too small for this the apical system was treated whole. Each genital plate was rubbed down on fine sand paper (emery paper is unsuitable as it stains the plates) until the outer surface was removed, and the rings showing to the best advantage. This stage could only be found by practice, and varied in different individuals, but as four genital plates were available, one or more could be sacrificed in obtaining the optimum result. The plate was damped with alcohol and examined with a hand lens from time to time during the

grinding, and when finished, it was transferred for a few minutes to absolute alcohol, and then to xylol, and finally mounted face downwards on a slide with Canada balsam. When dry the plate could then be examined through the slide. The sets of four genital plates of from four to seven urchins could be mounted on one $3'' \times 1\frac{1}{2}''$ slide.

The genital plate is five-sided, with the genital pore normally situated

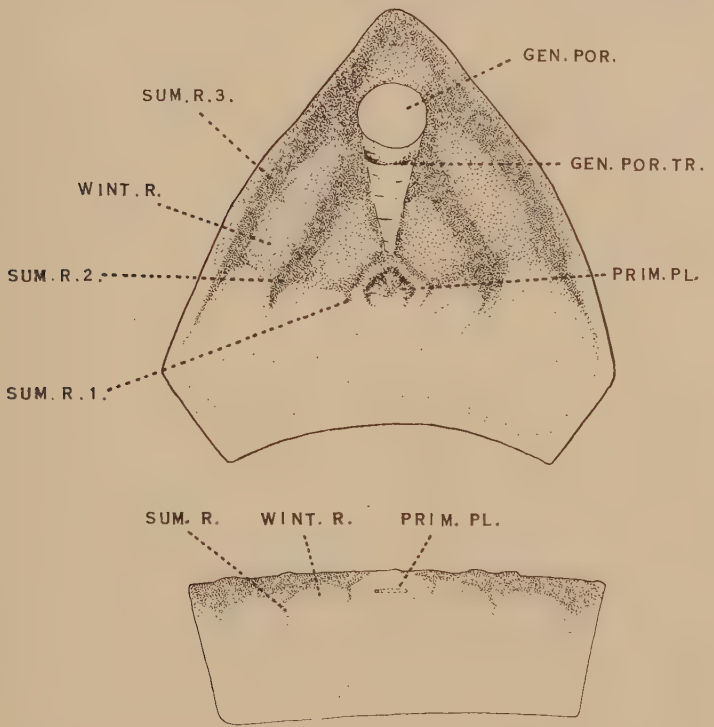

Fig. 5.—Genital plate showing three summer rings. A, horizontal section; B, vertical section. Sum. R., Summer ring. Wint. R., Winter ring. Gen. Por., Genital Pore. Gen. Por. Tr., Genital pore track. Prim. Pl., primitive, post-metamorphic plate.

at the apex (Fig. 5A). In transverse section (Fig. 5B) there is seen to be an external pigment layer which increases in depth towards the edge, and when being ground, this pigment layer is found to be considerably harder than the uncoloured layer beneath it. In the centre, and close to the surface is a thin plate which appears to be the first-formed post-larval genital plate. The depth of the pigment layer varies seasonally, giving rise to the structures referred to as annual rings. In some transverse sections these may be seen faintly indicating the successive stages of

growth of the sides and inner surface of the plate, and showing that the addition of new material to the plate has been almost entirely on these surfaces, and hardly at all on the outside. The surface of the plate is ground away until the deeper summer pigment rings stand out clearly between regions of white. Owing to the convexity of the shell, and to the deepening of the pigment layer peripherally, it is difficult to show all the rings to the best advantage in a horizontal cut, and it was

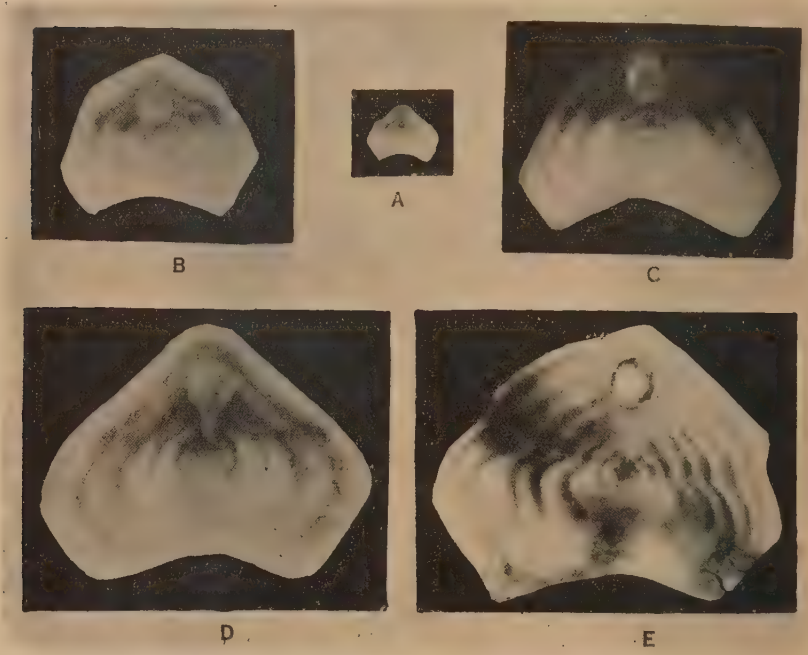

FIG. 6.—Horizontal sections of genital plates showing primitive plate in centre of each, and one (a), two (b), three (c), four (d) and six (e) dark winter zones outside. a. and d, Breast specimens, × 11. b. and c. Chickens specimens, × 15. e. Breakwater specimen. × 11.

therefore found expedient to grind the plate more deeply at one end, thus showing each ring clearly at some point in its course (Fig. 5A). The genital pore moves outwards in the plate with increasing size, and the space behind it is filled in with calcareous deposit, frequently unpigmented, which appears as a wedge-shaped track behind it. Abnormalities of the genital pore were fairly common. Sometimes it was situated to one side of the plate, and more rarely at the wrong end. More often there were two pores in the same plate, and in one case there were three. In these cases the track left by the pore showed that it was single at metamorphosis,

and divided later. A set of photographs of selected plates is shown in Figure 6. The primitive plate, when visible in the section, shows as a dark region in the centre of the plate, rather similar to the first summer ring, from which it must be distinguished. Between it and the first dark ring there is usually a narrow light zone, apparently corresponding to a period immediately after metamorphosis when the little urchin is either not feeding at all, or else not taking the same pigment-providing diet that it does soon after.

The seasonal identity of these rings is established from the examination of the relative width and colour of the outermost band at different

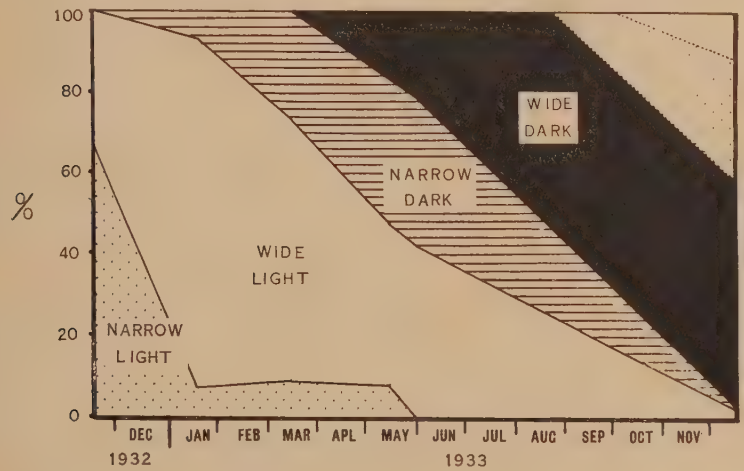

FIG. 7.—Seasonal changes in the colour of the outermost zone of the genital plates, from the Chickens.

seasons. The results obtained from a hundred and fifty-four urchins from the Chickens are shown in Figure 7. The period of new shell growth from March to July is the time of inception of a new dark summer zone in the shell, but apparently pigment can continue to be laid down in the shell by the inter-plate membranes, even when deposition of shell has ceased. This is to be expected, since newly formed calcium carbonate adsorbs pigments more readily than that which has been longer deposited. Individual variation is found, but it is not sufficient to confuse the zones of successive years, since by spring all urchins show an outer region of varying width of light shell. Similar seasonal changes were found on the Breakwater and Breast grounds.

The validity of using these rings for determining age is shown by comparison of the mean sizes for successive year groups so obtained, with the results obtained from the same material from the size distributions. In

Figure 8 this comparison is made for the Chickens, the curves being those drawn through the peaks of the size groups in Figure 3, and the rings show the means for the annual ring sizes, and the numbers of animals from which each was obtained. The numbers available were not very large,

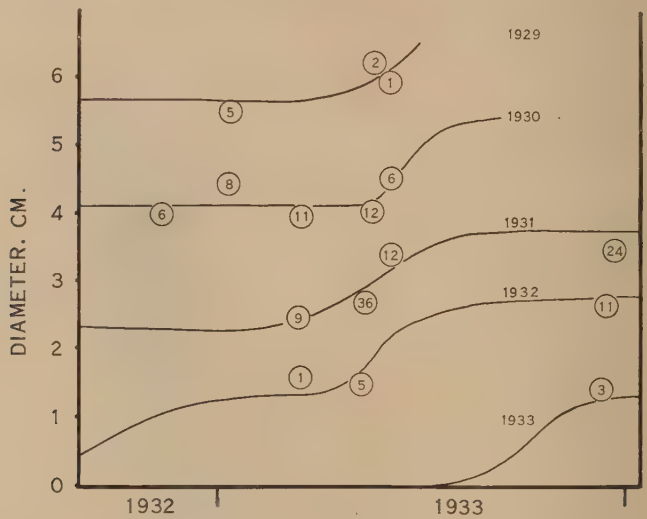

FIG. 8.—Growth of urchins from the Chickens, determined from size-distribution data (curves), and mean year-group sizes as determined from year rings (circles). The figures in the circles indicate numbers of specimens.

but the agreement is sufficiently good to indicate the validity of the method.

A possible error is introduced in the comparatively low percentage of the urchins in which the rings were readable. In the case of the Chickens samples 79% could be determined, but on the Breakwater the percentage fell to forty-six. If the individuals which are not readable have consistently a different growth rate from those which are readable, an error will certainly be introduced, but there is no evidence to suggest this, and the goodness of fit with results obtained from size groupings make it seem unlikely. At any rate, this is the best method of age determination at present available for the urchins. Attempts were made to keep urchins alive in a large cage on the Breakwater, but without success. In addition a method of attaching a numbered tag to the urchin with an elastic band proved impracticable.

The method of annual ring counting was applied to material from the Breakwater and Breast grounds, and the resulting growth rates are shown in Figure 10. There are no available size-distribution data for the Breast,

but for the Breakwater the results from the two methods are in substantial agreement as shown in the table below. It should be noted that the interval between the November–December and the March samples covers part of the growing period, with the result that the results for the latter

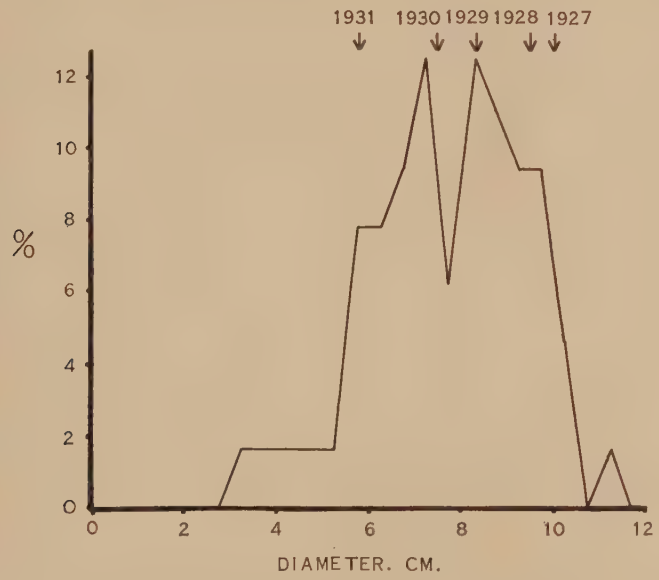

FIG. 9.—Size distribution for Keppel Pier, Millport, in February, 1934. Arrows show mean year-group sizes as indicated by year rings.

period are slightly larger than the former, especially in the case of the smallest sizes.

Diameter.	Year Group. 1927.	1928.	1929.	1930.	1931.	1932.
Nov.–Dec. ♂+♀	10·7	9·2	8·4	7·5	5·5	–
March ♂	–	–	8·4	7·7	6·6	–
♀	10·6	9·6	8·6	8·0	6·8	4·8

The only previous data on the growth of this species are those of Elmhirst (1922, p. 667), where the sizes of urchins for the Clyde are given at one, two, three and four years old as 4, 4–7, 7–9 and 9–11 cm. respectively. A sample of 64 urchins trawled by him off Keppel Pier in February, 1934, was examined, both for size distribution and annual rings, with the results shown in Figure 9 : 51 % of the urchins were readable. The mean year group sizes obtained from the annual rings are in

good agreement with those shown in the size distribution diagram, but indicate a complete absence of the first two year groups. Elmhirst assumed that the groups he found were composed of animals of 1, 2, 3 and 4 years old, and if this is the case, his results are in striking disagreement with those for Keppel. If on the other hand he was dealing with material from which the smallest sizes were missing, and he really had animals of 3, 4, 5 and 6 years old, his results would approximate better to those from Keppel. Such a deficiency of small sizes might have been due either to

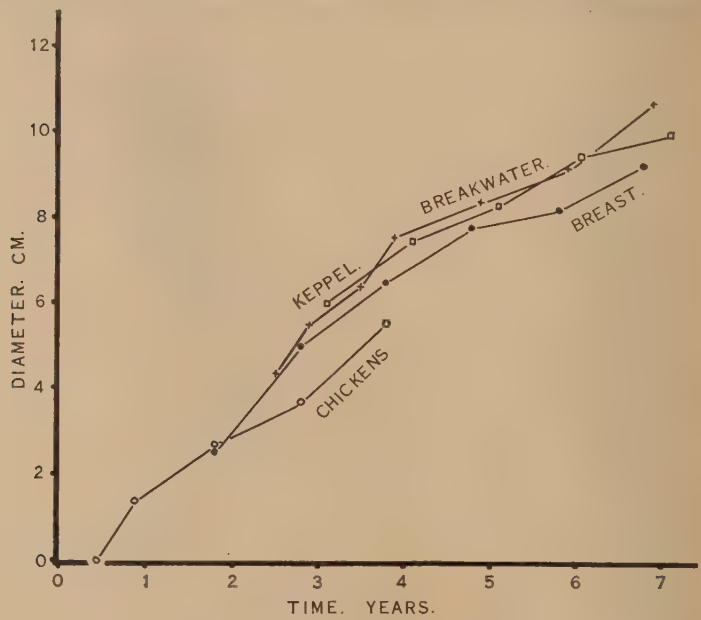

Fig. 10.—Growth of *Echinus esculentus*.

the selective action of the trawl with which they were taken, or to the result of previous unfavourable years.

The growths throughout life of typical urchins on the three Port Erin grounds, and at Keppel, are shown in Figure 10. In the case of the Chickens, on which seasonal observations had been made, the winter size is taken for comparison with the other grounds. As might be expected, growth is considerably slower on the Chickens ground than on any of the others; conditions there being presumably unfavourable. Growth on the Breast is more rapid, but slightly less so than on the Breakwater where, as reflected in the sizes of the gonads, there is considerably more available food than offshore. The Keppel sample shows a very similar rate to the Breakwater.

The largest urchin obtained from the Chickens had a diameter of seven centimetres, and was therefore probably four years old, but the usual size was considerably less than this. On the Breast a few specimens were taken up to 10 cm., but the usual limit was nine, corresponding to an age of six or seven years. The largest specimen from the Breakwater was 12·1 cm. in diameter, and probably seven or eight years old, although its rings were not countable. This was, however, exceptionally large, and the limit for ordinary specimens is about ten centimetres or six years. At Plymouth urchins over fifteen centimetres in diameter are taken, and it is hoped that it may be possible to determine the growth rate there also in the future.

I wish to acknowledge my indebtedness for assistance and suggestions in this work, to Prof. J. H. Orton, Dr. A. C. Stephen, Mr. R. Elmhirst and all the staff of the Port Erin and Plymouth laboratories.

SUMMARY.

1. *Echinus esculentus* was studied from four localities in the Isle of Man and from one in the Clyde.

2. The inshore races are thicker shelled and flatter, and on all grounds the urchins become taller with increasing age.

3. Size distribution measurements on the Chickens material showed a rapid growing period in the spring, followed by cessation of growth for the rest of the year. There is a correlation between amount of growth in a given year, and the sea temperature. On the Breakwater the males and females grow at slightly different times of year.

4. Concentric pigmented rings are demonstrated in the test, and are shown to be annual. They are used for estimating the growth rates on the different grounds, which are compared.

5. The duration of life is four to eight years, and perhaps more in the very large southern urchins.

REFERENCES.

BRUCE, J. R. 1928. Physical Factors on the Sandy Beach. Part I. Tidal, Climatic and Edaphic. Journ. Mar. Biol. Assoc., N.S., Vol. XV, No. 2, p. 535.

ELMHIRST, R. E. 1922. Habits of *Echinus esculentus*. Nature, Nov. 18.

MACBRIDE, E. W. 1914. Text Book of Embryology. Vol. I. Invertebrata. Macmillan & Co. London.

MOORE, H. B. 1934. A Comparison of the Biology of *Echinus esculentus* in Different Habitats. Part I. Journ. Mar. Biol. Assoc., N.S., Vol. XIX, No. 2.

THOMPSON, D'ARCY W. 1917. On Growth and Form. Cambridge University Press.

TABLE I.

RELATION OF THE VALUE OF $\sqrt[3]{\bar{V}^1}$ FOR A GIVEN VALUE OF D ON THE THREE PORT ERIN GROUNDS.

Breakwater.		Breast.		Chickens.	
D. cm.	$\sqrt[3]{\bar{V}^1}$ cm.	D. cm.	$\sqrt[3]{\bar{V}^1}$ cm.	D. cm.	$\sqrt[3]{\bar{V}^1}$ cm.
4·40	2·91	1·31	0·73	1·65	1·03
4·84	3·13	6·70	4·77	2·39	1·43
5·87	3·99	7·22	5·03	3·02	1·99
7·14	4·95	7·37	5·20	3·52	2·33
7·59	5·31	7·79	5·47	4·58	3·08
8·52	5·93	8·41	5·86	(5·63)	(3·96)
8·88	6·17	(9·33)	(6·38)		
9·37	6·57				
(10·2)	(7·15)				

Bracketed numbers are supported by six or less specimens.

TABLE II.

Size Distributions of "Chickens" Urchins for each Month given as Numbers in each 0·5 cm. Group (Test Diameters).

Month.	0·25	0·75	1·25	1·75	2·25	2·75	3·25	3·75	4·25	4·75	5·25	5·75	6·25	6·75	7·25	7·75	No. of specimens.
1931																	
May	–	–	–	–	2	8	5	11	11	3	1	1	–	1	–	–	43
June	–	–	–	4	8	8	9	13	10	–	1	–	1	1	1	–	56
1932																	
February	–	–	–	1	2	14	19	7	1	1	–	–	–	–	–	–	45
March	–	–	2	9	6	9	6	1	–	1	–	1	1	–	–	–	36
April	–	–	6	10	25	22	21	12	13	2	–	–	1	–	–	–	112
May	–	–	1	–	2	7	18	15	3	1	1	2	–	–	–	–	50
June	–	–	1	–	1	1	6	19	11	3	3	2	1	–	–	–	48
August	–	–	–	1	2	–	2	4	6	–	–	–	–	–	–	–	15
September	–	1	–	3	3	1	–	2	6	2	–	–	1	–	–	–	19
October	–	3	1	1	2	3	2	3	5	–	–	–	–	–	–	–	20
November	–	1	6	3	1	–	–	2	7	5	1	–	–	–	–	–	26
December	–	1	–	4	7	3	3	15	7	3	1	–	–	1	–	–	45
1933																	
January	–	–	–	1	2	3	7	11	12	8	2	1	1	–	1	–	49
March	–	–	–	2	8	8	8	14	16	1	1	–	–	–	–	–	58
April	–	–	–	–	–	5	1	5	9	2	–	1	–	–	–	–	23
May	–	–	2	5	12	19	24	8	1	3	3	2	2	1	–	–	82
December	–	–	2	–	4	11	8	10	6	–	–	–	–	–	–	–	41
1934																	
January	–	–	–	2	13	28	22	8	1	1	–	–	–	–	–	1	76
March	–	–	–	3	8	6	5	–	1	–	2	–	–	–	–	–	25
April	–	–	–	2	16	6	12	2	–	1	–	1	–	–	–	–	40
																Total	909

TABLE III.

Monthly Mean Temperatures for Port Erin Bay (Surface, 9 a.m.). As given by Bruce (1928), from an analysis of 25 years' results, and the variation of the monthly means from the above in the years 1930–33.

Month.	Mean. °C.	1930.	1931.	1932.	1933.
January	7·78	+0·8	+0·3	+1·4	+0·8
February	7·08	0·0	0·0	+0·9	+0·2
March	6·78	+0·1	−0·8	+0·7	+0·5
April	7·43	0·0	−0·5	+0·6	+0·8
May	8·97	−0·1	−0·5	+0·1	+0·5
June	10·94	+0·5	−0·1	+0·3	+0·8
July	12·77	+0·3	−0·4	+0·5	+1·2
August	13·76	0·0	−0·3	+0·6	+1·1
September	13·32	+0·4	−0·1	+0·6	+1·4
October	12·29	+0·2	−0·9	−0·2	+1·1
November	10·44	+0·4	+1·1	+0·2	+1·0
December	8·74	+1·1	+1·4	+0·9	+0·8

TABLE IV.

Size Distribution on the Breakwater in November–December, 1933, and in February, 1934.

Expressed as percentages in two millimetre groups. November–December sample, 149 urchins; February sample, 276 urchins.

Diameter.	% Nov.–Dec.	Feb.
4·9	1·3	0·0
5·1	1·3	1·1
5·3	0·0	0·3
5·5	0·7	1·1
5·7	2·7	1·1
5·9	0·0	1·4
6·1	0·7	1·4
6·3	0·0	2·2
6·5	2·0	3·3
6·7	2·0	4·0
6·9	6·7	4·7
7·1	1·3	4·0
7·3	5·4	1·8
7·5	10·0	7·2
7·7	3·4	6·2
7·9	5·4	5·1
8·1	7·4	6·5
8·3	4·7	4·0
8·5	13·4	10·5
8·7	6·0	5·8
8·9	4·7	7·2
9·1	4·0	6·5
9·3	3·4	4·4
9·5	4·7	2·5
9·7	4·7	2·2
9·9	0·7	2·9
10·1	1·3	1·4
10·3	0·7	0·0
10·5	0·7	0·0
10·7	0·7	0·3
10·9	0·0	0·3

TABLE V.

Size Distribution on the Breakwater in March, 1934, Expressed as Percentages in 3 mm. Groups.

Total 85 males and 75 females.

Diameter.	Males.	Females.
4·45	0·0	0·0
4·75	0·0	2·7
5·05	0·0	1·3
5·35	0·0	0·0
5·65	0·0	0·0
5·95	1·2	1·3
6·25	2·4	2·7
6·55	3·5	4·0
6·85	2·4	9·3
7·15	4·7	0·0
7·45	8·2	5·3
7·75	8·2	12·0
8·05	11·8	17·3
8·35	20·0	8·0
8·65	16·5	14·7
8·95	7·1	9·3
9·25	4·7	1·3
9·55	3·5	5·3
9·85	2·4	2·7
10·15	2·4	0·0
10·45	1·2	2·7
10·75	0·0	2·7
11·05	1·0	0·0

TABLE VI.

Size Distribution of Male Breakwater Urchins, 1931–32. Expressed as percentages in 3 mm. groups.

Diameter.	April.	May.	June.	July+ Aug.	Oct.+ Nov.	Jan.	Feb.+ March.
5·65	0·0						
5·95	1·6						
6·25	3·2		0·0	0·0			
6·55	4·8	0·0	2·8	4·6	0·0		0·0
6·85	4·8	1·6	2·8	0·0	7·5	0·0	5·7
7·15	6·5	6·5	0·0	4·6	9·5	13·3	7·1
7·45	4·8	1·6	2·8	0·0	11·9	16·7	7·1
7·75	14·5	11·3	2·8	0·0	4·8	13·3	18·6
8·05	16·1	9·7	2·8	4·6	16·7	26·7	11·4
8·35	11·3	12·9	11·1	13·6	7·1	6·6	8·6
8·65	11·3	17·7	16·7	18·2	14·3	10·0	11·4
8·95	9·7	9·7	11·1	9·1	9·5	6·6	7·1
9·25	6·5	4·8	0·0	4·6	7·1	3·3	8·6
9·55	3·2	8·1	19·4	9·1	9·5	0·0	1·4
9·85	0·0	4·8	5·6	13·6	0·0	0·0	0·0
10·15	1·6	6·5	13·9	4·6	2·4	3·3	1·4
10·45	0·0	1·6	5·6	9·1	0·0	0·0	0·0
10·75		0·0	2·8	4·6			
11·05		3·2	0·0	0·0			
11·35		0·0					
No. of urchins	62	62	36	22	42	30	70

TABLE VII.

SIZE DISTRIBUTION OF FEMALE BREAKWATER URCHINS, 1931–32.

Expressed as percentages in 3 mm. groups.

Diameter.	April+ May.	June.	July+ Aug.	Oct.+ Nov.	Jan.	Feb.	March.
5·65	0·0						
5·95	1·3						
6·25	4·0						
6·55	0·0			0·0	0·0		0·0
6·85	4·0		0·0	2·3	5·0		2·9
7·15	2·7	0·0	1·2	9·3	0·0	0·0	11·8
7·45	5·3	1·6	3·5	9·3	15·0	20·8	8·8
7·75	10·8	0·0	5·9	11·6	10·0	20·8	11·8
8·05	13·3	3·3	9·4	14·0	10·0	8·3	14·7
8·35	5·3	6·6	9·4	4·7	30·0	12·5	2·9
8·65	12·0	23·0	16·9	30·2	0·0	8·3	11·8
8·95	8·0	6·6	10·6	11·6	15·0	8·3	5·9
9·25	8·0	8·2	15·3	2·3	5·0	4·2	11·8
9·55	4·0	21·3	11·8	4·7	0·0	12·5	11·8
9·85	2·7	8·2	5·9	0·0	0·0	4·2	2·9
10·15	10·8	8·2	2·4		5·0	0·0	0·0
10·45	2·7	4·9	4·7		5·0		2·9
10·75	1·3	3·3	1·2		0·0		0·0
11·05	4·0	1·6	1·2				
11·35	0·0	0·0	0·0				
11·65		3·3	1·2				
No. of urchins	75	61	85	43	20	24	24

Edwardsia callianthus spec. nov. A New British Species from Menai Straits.

By

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Herdman Scholar and Forbes Exhibitioner in the Department of Zoology, Liverpool University.

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INTRODUCTION.

WHILE collecting in Menai Straits in July, 1932, Professor Orton found three specimens of an Edwardsia-like anemone. They were kept alive in finger bowls for several months; and when they were examined in the following November about two dozen young anemones were discovered with them. These Professor Orton kindly showed to me, and suggested a further examination of the young and adults.

On investigation it was found that the adults possessed all the characters of the genus Edwardsia as defined by Carlgren and Stephenson (**1**) but that they differed from other known British members of the genus in having more than sixteen tentacles and a scattered arrangement of the nemathybomes, which as well as being scattered are not visible in the living animal, at least under a binocular microscope.

Several unsuccessful attempts to collect more specimens of this new anemone were made by H. A. Cole, M.Sc., of the University College, Bangor, and myself. In March, 1933, however, we were successful, and since then I have obtained approximately thirty specimens at various times of the year.

THE MENAI EDWARDSIA
EDWARDSIA CALLIANTHUS SPEC. NOV.

Diagnosis.

Physa well developed, perforated by numerous pores. Scapus with a fairly thick periderm. Nemathybomes not visible in the living anemone, numerous, small, irregularly scattered and usually single. Tentacles sixteen to thirty-three in number. They are arranged in three cycles, an exocœlic and an outer and inner endocœlic cycle. In all the specimens so far examined with twenty or more tentacles there were only four tentacles in the inner endocœlic series, and these belonged, wherever their arrangement was fully ascertained, to the directive endocœles, and to the dorso-lateral primary endocœles. The tentacles were therefore arranged as follows : 4, 6, 10=20 ; 4, 7, 11=22 ; 4, 8, 12=24 ; 4, 9, 13=26 ; 4, 12, 16=32, etc. Pennons of the macrocnemes in the upper part of the fertile region with about twenty-five to thirty folds, some of which are branched, particularly those in the outer part of the pennon. Outer lamella of the mesenteries attached to the pennons some distance from their outer edge. Parietal muscles, in the upper part of the fertile region, triangular in transverse section, with a thick central stem and about seven fairly stout branches on either side, some of which may bifurcate. Lower down the column the parietal muscles are more elongate and their branches not so spreading. Their extension on to the column wall is about as usual. Microcnemes present but small. Unexploded nematocysts of the tentacles 14–29×2–5·2μ, those of the scapulus 8–16×2–3·5μ and of the nemathybomes 26–71·7×2·1–7·3μ. Nematocysts of the actinopharynx of two kinds, a small variety, 7–11×1–2·5μ with the basal part of the thread visible in the unexploded capsules and a large variety 17·7–36·5×2–4·7μ with the thread entirely concealed. Tentacle spirocysts 11·4–27×2–7·3μ. A single ventral siphonoglyph is present.

(*a*) *Colour.*

Physa :—

Transparent and colourless.

Scapus—periderm :—

Rusty red or sandy-brown, frequently greyish black towards the oral end.

Tentacles :—

Translucent and sometimes colourless, pale brown or pink. Pattern present or absent, when present it is either a small white fleck at the tip or a fine white continuous or discontinuous line along their length.

Disc :—

Transparent and colourless, grey or brown with a chalky or creamy white powdering developed as alternating broad and narrow triangles opposite the endocœlic and exocœlic tentacles respectively and with their apices directed towards and sometimes extending on to the top of the hypostome where they may then form a white encircling ring just beneath the lip lobes. The mesenterial insertions show as grey or brown radiating lines between the white triangles.

Scapulus :—

Transparent and colourless sometimes with two broken bands of chalky or creamy white, one at the bases of the tentacles and the other about half-way down its length. The former band is often absent, the latter is usually present and frequently developed as a series of deep or shallow inverted U-shaped markings but sometimes as irregular rectangles, one on each scapular ridge. The lip lobes and the actinopharynx are usually dull orange but occasionally pink. The latter shows through the scapulus as a coloured column.

(*b*) *Size.*

The length of the column in mature specimens is about 4–6 cm., the breadth, 0·3 cm.

(*c*) *Occurrence.*

The species is so far known only from Church Island, Menai Straits. It is found at low water of Spring tides or just below this level, in sandy areas mixed with fine gravel and small stones (see page 141).

Description.

(*a*) *External Features.*

The body is vermiform in extension. It is clearly divisible into a physa, scapus and capitulum.

The *physa*, when expanded, is a thin-walled bulb with eight longitudinal lines which represent the insertions of the macrocnemes. Sometimes it shows one or two circular constrictions. It may be completely collapsed by the gradual invagination of its central part so that it appears as a flat disc with eight furrows radiating from a central pit. Its surface is covered with microscopic adhesive rugæ. At the aboral end there is a

terminal pore with several other smaller apertures (9 and 12 in two sectioned specimens) arranged in a circle round it.

The *scapus* is very contractile. When expanded it is long and slender. It is widest just below the middle and narrows at either end. It is enveloped in a tough leathery periderm which is in close contact with the surface of the body but may be readily peeled off. Masses of minute

Fig. 1.—*Edwardsia callianthus* showing the sandy investment. This specimen was semi-narcotised and shows the physa in a collapsed state. × about 1½. Photograph by P. Bond.

oval greenish brown bodies, sand grains and a few diatoms adhere to its outer surface and give it a fine transversely furrowed appearance (see Fig. 1). The thickness of the accumulated particles varies in different specimens; where they are plentiful the furrows are quite distinct, where they are less abundant the furrows are hardly perceptible. Overhanging the junction between the scapus and the physa there is often a particular accumulation of sand which forms an irregular frill. The mesenterial insertions are not visible in fully expanded specimens except in those with a very thin investment. In a semi-contracted individual they show as furrows in the anterior half of the scapus while in a fully contracted animal the furrows may appear along its entire length.

The distal portion of the column, as in other Edwardsias, consists of two

regions which are anatomically distinct from one another, the capitulum proper, a narrow zone just beneath the tentacles, and the scapulus, a more extensive region between the capitulum and the scapus, and belonging morphologically to the latter.

The *capitulum* is externally indistinguishable from the scapulus and

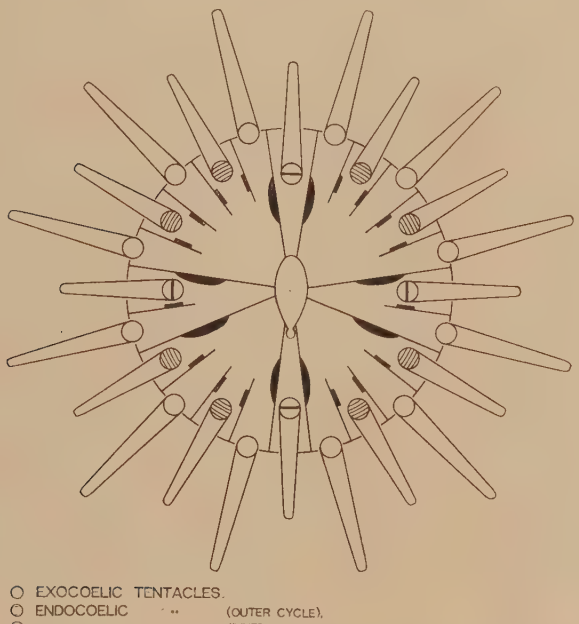

FIG. 2.—Diagram showing the arrangement of the tentacles in a specimen of *E. callianthus* in which their number was twenty-four. (The microcnemes do not have retractor muscles, they are figured in the diagram to show how the mesenteries pair.)

bears from sixteen to thirty-three tentacles. In thirty-one specimens their numbers were as follows :—

No. of tentacles	16	17	20	22	23	24	26	33
No. of individuals	2	3	8	7	1	6	2	2

The anemones with sixteen tentacles were doubtless young ones, they were smaller than the rest. The tentacles are arranged in three distinct cycles ; there is an outer exocœlic series, the members of which alternate with the tentacles of an inner endocœlic series in which two cycles, an inner and an outer, are distinguishable (see Fig. 2). In a fully expanded anemone the exocœlic tentacles are usually longer than the endocœlic. It is nevertheless often impossible to see any difference between

FIG. 3.—A side view of the disc and upper part of the scapulus of *E. callianthus* to show the pattern.

FIG. 4.—Diagram of the pattern on the disc and on the upper part of the scapulus just beneath the tentacle bases of *E. callianthus*. (The tentacles are not drawn.)

their relative lengths and sometimes the exocœlic ones appear shorter, particularly in a partially expanded individual. The inner tentacles are frequently held in a vertical position with their tips bent inwards while the exocœlic ones are turned back to lie either flat on the substratum or against the scapulus. They are all very contractile, and continually in motion. They are long, slender and tapered towards the distal extremity, where they terminate bluntly. The endocœlic tentacles are however somewhat thicker than the exocœlic ones. They are all usually the same colour, either transparent and colourless, pale brown, pale or deep pink, but in one individual the exocœlic series was brown and the endocœlic series pink. In a few anemones there was no colour pattern, in others some or all the tentacles showed a small white fleck at the tip (see Fig. 3), while others had a fine white continuous or discontinuous line along their length. Any or all of these markings may be present in the one individual.

The arrangement of the tentacles seems to follow a fairly definite plan. Seven living and several preserved specimens which I have examined in detail each possessed more than twenty tentacles, four of which belonged to the inner cycle of the endocœlic series ; they were the dorsal and ventral directive tentacles, and a tentacle in each dorso-lateral primary endocœle. Figure 2 shows the usual arrangement of the tentacles when their number is twenty-four. Two anemones, each with twenty-two tentacles, showed a similar arrangement except that in one of the dorso-lateral macrocœles there was only one endocœlic and two exocœlic tentacles. In Figure 4, representing a specimen with twenty tentacles, the same arrangement is also evident but in both the dorso-lateral macrocœles there is only one endocœlic and two exocœlic tentacles. Professor Stephenson (2) has figured the tentacle plan of a specimen with thirty-three tentacles which was sent to him. Here, also, the inner cycle of the endocœlic series contains four tentacles which are situated in the same positions as in the specimens which I have examined.

The hypostome forms a prominent cone with eight distinct protrusible lip lobes surrounding a slightly elongated mouth.

The disc pattern as illustrated in Figure 4 is typical of all the specimens which have been examined, irrespective of their tentacle number. Minor variations from this diagrammatic representation do, however, occur. The intensity and extent of the pattern varies in different individuals. In some the whole disc is white except for the grey or brown lines which mark the insertions of the macrocnemes and microcnemes, in others the pattern is not so extensive, being developed only at the foot of each tentacle and as a narrow streak along the insertion of each mesentery. It becomes very feint when the animals are kept in captivity. The white encircling ring beneath the lip lobes may be present or absent.

The *scapulus* is a short region about 5 mm. long. The interseptal areas appear as eight broad ridges which are separated from one another by the insertions of the macrocnemes. When slightly contracted it shows a series of very fine white transverse lines. Figure 3 shows the pattern at the tentacle bases developed as alternating triangles and inverted U-shaped markings, and Figure 1 the band of inverted U-shaped markings mid-way between the bases of the tentacles and the scapus. The tentacles may be completely withdrawn by the gradual invagination of the capitulum and the scapulus into the scapus. The anterior end of the scapus with its investment is also frequently invaginated but sometimes the investment projects as a short free tube.

(*b*) *Anatomy.*

The material for anatomical study was narcotised with menthol, fixed in Bouin's fluid, stained in bulk with borax-carmine and embedded by the usual xylol-wax method. The sections were counter-stained with picro-indigo-carmine. A detailed account of the anatomy is inessential, since in general it is similar to that of other species of Edwardsia.

The ectoderm of the *physa* is columnar and contains numerous gland cells. They secrete an adhesive mucus which attaches the anemone to the substratum. The structure of all the apertures of the physa is the same. Each is developed as a gap in the body wall and is surrounded by circular muscles. Projecting through each aperture there is an epithelial plug which is pierced by a canal. Sometimes the plug is inwardly directed towards the cœlenteron, at other times outwardly directed. Carlgren (**3**) has described similar epithelial plugs in the physa of *E. vitrea* and *E. vegæ*. He suggests that in the former they function as movable stoppers and that the observed difference in their position depends upon the state of contraction of the physa. This same explanation is probably also true for *E. callianthus*. The possibility of a change in the position of the plug makes it impossible to say whether the apertures are developed as ectodermal invaginations or as endodermal evaginations.

The *scapus* has a well-developed periderm. The mesoglœa is thick and laminated, and contains a large number of nemathybomes. They are not visible in the living animal even when the investment is removed, because they are sunk into the mesoglœa and not mounted on prominent tubercles as in certain other species of Edwardsia. Their distribution may be seen if a portion of the wall is removed, cleared and mounted in glycerine (see Fig. 5). They are absent from all other parts of the body, although a few may occur in the transitional region between the scapus and the physa. Their structure is as usual. Each is a spherical cavity which opens on to the ectoderm by a narrow aperture. The capsules of the nemathybome nematocysts have a wide range in size (see Table I). Their

shape is also variable; some are bow-shaped, others straight, but all degrees of curvature are met with between these two extremes. They are all slightly broader at one end. It is possible that the variation in shape depends upon their position in the nemathybomes, the curved ones lying against the walls of the cavity, the straight ones in the middle; but both directed towards the aperture in such a position that they can be exploded.

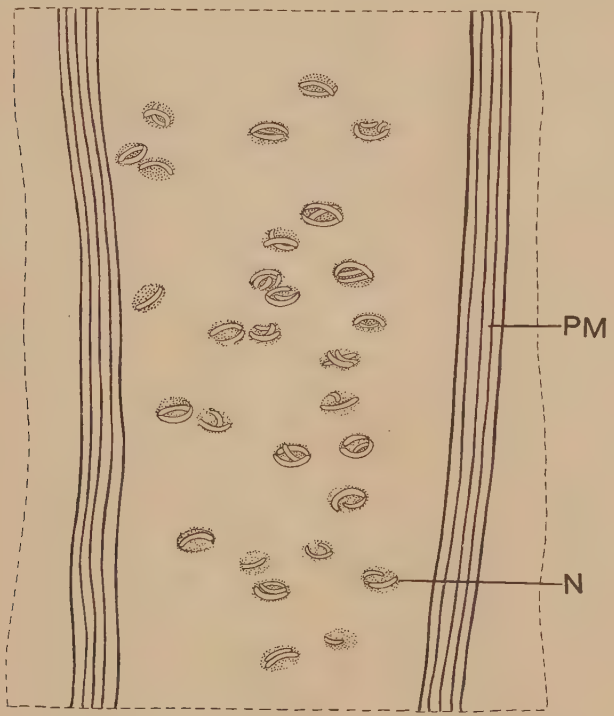

FIG. 5.—Surface view of a portion of the scapus wall cleared in glycerine to show the arrangement of the nemathybomes. N. nemathybome, P.M. parietal muscle. × 80.

The anatomy of the *scapulus*, *capitulum* and *tentacles* agrees with that of other species of Edwardsia (**3**). The actinopharynx is about the same length as the scapulus. It is lined with ciliated epithelium which is raised into eight broad folds, one opposite the attachment of each macrocneme. Between each pair of folds there is a deep furrow except between the folds corresponding to the dorsal directives, which are only indistinctly separated from one another. A transverse section through the actinopharynx is very similar to that of *E. callimorpha* (Fig. 15, **4**). The ventral siphonoglyph is structurally scarcely distinguishable from the remaining

FIG. 6.—A transverse section of a retractor muscle in the upper part of the fertile region of *E. callianthus*. (The mesoglœa is shown as black and the fertile and cnidoglandular areas are stippled.) × 186.

FIG. 7.—A transverse section through a retractor muscle in the region of the actinopharynx of *E. callianthus*. (The mesoglœa is shown as black.) × 189.

furrows. Small nematocysts are present in the epithelium of the actinopharynx and the ectoderm of the scapulus, and along with the spirocysts they are very abundant in the tentacles. A comparison of their sizes is given in Table I.

TABLE I.

A Comparison of the Size of the Nematocysts from Various Regions of the Ectoderm of *E. callianthus*.

(The individuals which were used were 4–6 cm. long. The measurements were made of living capsules unexploded, unstained and mounted in sea-water unless otherwise stated. The width measurements were made half-way down the capsules.)

	No. of individuals.	Number and condition of the cells when measured.	Range in size of the cells in μ.	Average size of the cells in μ.
Nematocysts from the tentacles	2	50	14–28 × 3·1–5·2	23·5 × 3·8
	1	14 (exploded)	16·6–29·0 × 2·0–3·6	24·3 × 3·1
	1	32 (preserved in 5% formalin)	14–29 × 2–4	24·4 × 3
Nematocysts from the nemathybomes	3	77	34·3–71·7 × 2·1–7·3	48·6 × 4·9
	1	51 (preserved in 5% formalin)	26–68·6 × 3–5·1	44·3 × 3·9
Nematocysts from the actinopharynx	1	21 (large variety preserved in 5% formalin)	18–36·5 × 2–4	27·7 × 3·1
		22 (small variety preserved in 5% formalin)	7–11 × 1–2·5	9·2 × 1·8
	1	27 (large variety)	17·7–35·3 × 2–4·7	27·4 × 3·4
Nematocysts from the scapulus	1	24	9·4–12·4 × 2·1–3·1	10·7 × 2·6
	1	29 (preserved in 5% formalin)	8–16 × 2–3·5	11·8 × 2·7
Spirocysts from the tentacles	3	59	11·4–25·5 × 2·5–7·3	19·6 × 4·3
	1	20 (preserved in 5% formalin)	11·5–27 × 2–4	18·3 × 3

There are eight typical Edwardsian *mesenteries* extending throughout the entire length of the column, and a series of microcnemes which are confined to the capitular region. The latter, as usual, are small lamellæ each with a mesoglœal core and without either retractor muscles or mesenterial filaments. Figure 2 shows four microcnemes pairing with the lateral macrocnemes, and an additional pair corresponding with each endocœlic tentacle.

The structure of the perfect mesenteries is typical. The form of the

FIG. 8.—A transverse section of a retractor muscle below the fertile region of *E. callianthus*. (The mesoglœa is shown as black.) × 186.

FIG. 9.—A transverse section of a parietal muscle in the upper part of the fertile region of *E. callianthus*. (The mesoglœa is shown as black.) × 672.

pennons and their retractor muscles as seen in transverse section gradually changes from the oral to the aboral end of the animal. In the upper part of the fertile region and in all parts anterior to it, the retractor muscles have about twenty-five to thirty folds, some of which branch two or three times (see Figs. 6 and 7). In the reproductive region the muscle processes are better developed than elsewhere and give the retractor a distinctly feathery appearance. Below the reproductive tract, the folds gradually decrease in number and the mesoglœal core thickens (see Fig. 8). Figure 9 represents a parietal muscle from the upper part of the fertile region. Although the form of the retractor and parietal muscles is a useful character for the identification of species belonging to the genus Edwardsia, Carlgren (**3**) has shown that their form varies in individuals of the same species from different localities. The ciliated tracts are discontinuous. Below the fertile region the inner part of each mesentery is drawn out into a thin lamella as in the species described by Faurot as *E. beautempsii* (Plate I, Fig. 3, **5**).

Habitat.

The shore off Church Island, Menai Straits, is rocky with large stretches of fine mud and sand. In certain small areas, the sand just beneath the surface is a distinct rusty-red-brown in colour and is mixed with gravel and small stones. It was in sheltered ground of this type that most of the anemones were discovered. They may be found at low water of

TABLE II.

Analysis of the Sand Inhabited by *E. callianthus* at Church Island.

Size of mesh through which the particles were passed and on which they were collected.	Sample 1 % dry weight.	Sample 2 % dry weight.	Sample 3 % dry weight.	Designation.
On 15 mm.	0	0	5·94	Stones.
Through.				
15 mm. on 5 mm.	4·83	3·98	5·0	Coarse gravel.
5 mm. on 4 mm.	2·90	1·85	2·46	Medium and Fine ,,
4 mm. on 3 mm.	2·60	2·06	1·95	
3 mm. on 2 mm.	2·93	2·21	2·43	
2 mm. on 1 mm.	7·59	5·24	6·31	Coarse sand.
1 mm. on 0·5 mm.	9·83	12·75	8·42	Medium ,,
0·5 mm. on 0·25–0·35 mm.	26·11	16·67	20·09	Fine sand and silt.
0·25–0·35 mm. on 0·07–0·1 mm.	33·61	40·62	34·63	
0·07–0·1 mm. on filter paper	10·10	14·59	12·72	

Spring tides, but the majority live in regions unexposed by the average tides. The analysis of three separate samples of sand in which the anemones were living is given in Table II. The sand was passed through a series of zinc sieves with circular perforations ranging from 15 mm. in $\frac{1}{2}$-mm. sizes down to 0·5 mm. The residue collected on the 0·5-mm. sieve

was then passed through a silk mesh of 0·25–0·35 mm. and collected on a silk mesh of 0·1 mm. The sand and silt which passed through the 0·1-mm. mesh was collected on weighed filter papers. The graded samples thus obtained were dried in the sun, weighed, and their percentage of the total sample determined. Following Allen's nomenclature (**6**) the habitat of *E. callianthus* may be defined as mainly fine sand with a small proportion of medium and course sand and gravel.

Habits.

E. callianthus lives in narrow burrows a few inches below the surface of the sand. It appears to be gregarious; sometimes two individuals inhabit the same burrow. On one occasion twenty specimens were collected from three forkfuls of sand and on another occasion seven from one forkful, yet none were discovered in the immediate neighbourhood. The individuals are usually free, but the physa may be attached to a pebble.

The anemones live quite well when kept in the laboratory in finger bowls containing a fairly thick layer of sand. They frequently adhere firmly to the bottom of the vessel by means of the physa. Sometimes they burrow in the sand, at other times they are free on the surface. The physa is usually regarded as the burrowing organ of the Edwardsias, but I have seen one specimen of *E. callianthus* burrowing with its tentacles. It was lying fully expanded on the sand and commenced to burrow slowly forwards with its tentacles extended and actively moving. They seemed to be the principal agents in moving the sand. One anemone was found in its natural habitat lying free on the sand beneath the water. It was perfectly healthy and lived in the laboratory for some time afterwards.

On several occasions complete narrow rings of investment were discovered in the bowls in which the anemones were living. They were probably cast off from the aboral end of the animal when the physa contracted, since the covering is always more irregular here than elsewhere.

During the first few weeks of captivity the anemones quickly contract at the least vibration; later they are much less sensitive and remain expanded for long periods. A similar habit is recorded for *E. callimorpha* (under the name *claparedii*, **7**).

Larvæ and Young Individuals.

Relatively little is known about the early larval stages of the Edwardsidæ, and the records which are available are in many cases very brief. Owing to an insufficient supply of material it has only been possible to make a few observations on the young of *E. callianthus*, but they are of interest for comparison with other members of the family.

FIG. 10.—*E. callianthus*, a few days old. (Length of column 1·6 mm.)

FIG. 11.—*E. callianthus* eleven days older than the specimen in Fig. 10. (Length of column 1·9 mm.)

E. callianthus is sexually mature about November and December. Two specimens examined in December, one in 1932 and the other in 1933, contained fully developed eggs and another had a ciliated larva in the cœlenteron. Young individuals were produced in the laboratory about November 17th from adults which had been collected the previous July. The young were about 1·5 mm. long when they were discovered attached to the bottom of the finger bowl and to the surface of the adult. It is however possible that they were liberated a few days earlier. Some were in the late planula stage, others showed eight rudimentary tentacles and the body divisible into a physa and scapus, but there was no sandy investment (see Fig. 10). The physa was colourless and transparent, the scapus pale pinkish brown with the insertions of the macrocnemes showing clearly along its length. There were three tentacles on either side of the mouth and one at either end which was a little shorter than the rest. The hypostome formed a prominent cone. The discovery of young individuals —at the most only a few days old—in a relatively advanced stage of development and the presence of a ciliated larva in the cœlenteron of one adult strongly suggests that *E. callianthus* is viviparous. Viviparity has not so far been recorded in any other species of Edwardsia, although it is suspected in *Milne-Edwardsia carnea*. As growth proceeds the tentacles elongate and two fairly distinct cycles of four long ones alternating with four short ones are frequently distinguishable (see Fig. 11).

The anatomical features of the young are similar to those described for other members of the genus (**8**, **9** and **10**). The youngest larvæ which were sectioned were in the late planula stage, the oldest, with eight rudimentary tentacles. In the former the blastocœle was almost filled with cells in which eight macrocnemes were recognisable. Each mesentery had a thin core of mesoglœa and showed developing retractor muscles. The intermesenterial spaces arise by the gradual disappearance of some of the central cells, those which remain form the endoderm of the scapus wall and mesenteries. The ventro-lateral mesenteries are the largest, and the first to develop mesenterial filaments. The ectoderm of the entire body wall and the epithelium of the actinopharynx is glandular. It contains a large number of clear vesicles. Nemathybomes were not developed in any of the larvæ which were investigated.

When the young were examined on November 30th, one showed a rudimentary ninth tentacle. Their development has not been followed further than this stage.

CONCLUSION.

One cannot conclude an account of *E. callianthus* without referring to its close resemblance to the anemone described by Dixon (**11**) as *E. timida*.

The external features and the colouring of these two anemones are remarkably similar. Carlgren (**1**) has recently investigated the anatomy of some specimens of "*E. timida*" sent to him by Dixon, and as a result of his researches has renamed it *Milne-Edwardsia dixonii*. A comparison of its anatomical features with those here described for *E. callianthus* shows that these two anemones are quite distinct; nemathybomes, for instance, are absent from *M. dixonii*, whereas they are present in *E. callianthus*. The close resemblance between their external features has nevertheless led Professor Carlgren to suggest (letter to the author) that Dixon possibly confused two species, the one a real Edwardsia and the other a Milne-Edwardsia. Professor Stephenson (**2**, p. 403) has followed up this suggestion, and finds that not only does Dixon's description probably cover two species, but possibly three. Only one of these (*M. dixonii*) has been anatomically described; and it is impossible to be certain about the others without obtaining material from Dixon's original locality. In any case, Dixon's use of the name *E. timida* was unjustified, so that whether or no *E. callianthus* was one of the forms included in his description, it requires a new name and is here characterised as *E. callianthus* spec. nov.

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REFERENCES.

1. Carlgren, O., and Stephenson, T. A. The British Edwardsiidæ. Journ. Mar. Biol. Ass., N.S., Vol. XV, No. 1, 1928.
2. Stephenson, T. A. The British Sea Anemones, Vol. II, London, 1934.
3. Carlgren, O. The Danish Ingolf Expedition. Vol. V, 9, Actinaria, Part 1, Copenhagen, 1921.
4. Stephenson, T. A. The British Sea Anemones, Vol. I, London, 1928.
5. Faurot, L. Études sur l'anatomie, l'histologie et le développement des Actinies. Archiv. de Zool. Exp. et Gén., 3me Sér., Tome III, Paris, 1895.
6. Allen, E. J. On the Fauna and Bottom Deposits near the Thirty Fathom Line from the Eddystone Grounds to Start Point. Journ. Mar. Biol. Ass., N.S., Vol. V, 1897.

7. Walton, C. L., and Rees, O. M. Some Rare and Interesting Sea Anemones from Plymouth. Journ. Mar. Biol. Ass., N.S., Vol. X, No. 1, 1913.

8. Marks, E. L. Selections from Embryological Monographs. Mem. Mus. Comp. Zool., Vol. IX, No. 3, Harvard, 1884.

9. McMurrich, J. P. Contributions on the Morphology of the Actinozoa. Journ. of Morphology, No. V, Boston, 1891.

10. Wietrzykowski, M. W. Récherches sur le développement de l'*Edwardsia beautempsii*. Bull. Int. Acad. Sci. Biologische Wissenshaften, No. 7 B, Cracovie, 1914.

11. Dixon, G. Y. Notes on two Irish specimens of *Edwardsia timida* (Quatrefages). Sci. Proc. Roy. Soc., Vol. V, Dublin, 1886.

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